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MONTHLY VARIATIONS IN MUSCULAR EFFICIENCY IN WOMEN

LILLIAN M. MOORE AND J. LUCILE BARKER

From the Department of Physiology, University of California, Berkeley

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The question of periodicity in the physiological processes of women has attracted desultory interest among scientists since the definite stand for periodicity taken by Jacobi (1). Hers was the first experimental work on the subject. Hollingsworth (2) many years later furnished extensive data but found only irregular variations with no evidence of a monthly rhythm. The modern trend is in accord with the latter conclusion that there is an absence of rhythm. However, a review of the recent work reveals a paucity of data collected from a small number of subjects who were tested at intervals of several days and for a period of only one or at most two months. It seemed as though the problem might be cleared up by testing a relatively large number of subjects as nearly daily as possible over a period including a large number of cycles so as to eliminate individual and chance variations. With this in view a series of experimental studies has been undertaken.

The purpose of the investigation reported in the present paper, the first of the series, was to determine the daily muscular efficiency of women over a period of several months in order to show whether it remains fairly constant or varies periodically; that is, whether there are regularly recurring periods of heightened and of lowered efficiency coincident with the monthly cycle.

The spring balance dynamometer designed by Martin (3) was used in making the tests. This method was chosen because it seemed successfully to eliminate errors, common to other dynamometers, such as

those due to practice and to variations in the voluntary effort of the subject to exert himself. Martin's test measures not the active pulling strength, but the breaking strength of the muscle; that is, the force necessary to overcome maximal resistance on the part of the subject rather than the utmost voluntary effort. In other words, it is a resistance test that is largely reflex in nature, and therefore not easily modified at will. Consequently the results would seem to be more constant and reliable than those obtained by other methods.

As originally practised by Martin, 44 muscle groups were tested, 12 on each arm and 10 on each leg. Later this was modified, only eight muscle groups being used: arm flexors, pectorals, thigh abductors and adductors of both sides of the body. This shorter test requires only about 4 minutes for performance. Martin has compared the relation of the total strength of the eight muscles to the entire strength of the body as found by the longer test. It represents 15 per cent of the entire strength for men and 14.2 per cent for women. Therefore, to calculate the entire strength or muscular efficiency of the body the sum of the determined strength of the eight muscle groups is multiplied by the reciprocal of 15 per cent, (6.67) for men, the reciprocal of 14.2 per cent, (7.04) for women.

The procedure described by Martin was adhered to in general. We found it necessary, however, to make a few minor modifications; as, for instance, in testing the thigh abductors and adductors, instead of having the foot held in position by an assistant it was suspended in the loop of a light rope attached to the ceiling just above the end of the table. This obviated the need of an extra assistant and also the personal variable resulting from the tendency of the assistant to aid in the resistance of the foot. Again, it was found that the friction of the strap on the arm in the flexor and pectoral tests caused soreness which at times invalidated the results. To eliminate this source of error a cloth pad was placed under the strap. It was noted that training was necessary to enable the operator—experimenter—to develop sufficient tension without jerking and to develop the same tension each time. To lessen variation from this source, three or four experienced operators only were entrusted with the testing, and whenever possible an individual subject was tested by the same operator each day of the series.

The investigation has extended over a period of 20 months with a total of thirty subjects. Unfortunately it was impossible to obtain subjects for whom unbroken daily records could be obtained. However, the tests were given daily whenever possible (in most cases this meant

every day except Sundays and occasional holidays and vacation periods) between the hours of 9 and 12, and under uniform experimental conditions. The majority of the subjects were teaching or attending classes in the laboratory, and the tests were, therefore, merely a part of the daily routine being given at the same hour each day for each individual.

The subjects with two exceptions were a representative group of college women, students and young instructors. All were in excellent health except no. 13. They were all the robust California type of outdoor girl who seldom finds it necessary to consider herself an invalid. Several were engaged in athletics; three were specializing in physical education; others took various kinds and amounts of exercise, as hiking, tennis, swimming, rowing, etc., and three were working for their board and room while attending college. Most of the subjects were upper classmen or graduates, and had, therefore, had the advantage of at least two years of physical training in college. With one exception they represented conditions of normal menstrual health, although several reported some degree of discomfort during menstruation. In no case, however, was college work remitted during this time, although most felt the need of a greater amount of sleep and rest. Twenty-five reported some slight decrease in physical and mental efficiency, two an increase, and one no change during menstruation.

Table 1 gives the number of subjects, periods during which tests were given, number of monthly cycles, and number of daily trials, total and individual.

Daily records were kept of the strength in gram millimeters of each of the eight muscle groups and of various conditions which might cause chance variations, such as number of hours sleep, condition of sleep, regularity of meals, physical, mental or psychic disturbances, and routine of the previous day.

Results and conclusions. The results of the daily observations were plotted for each individual with time in days as abscissae and total muscular strength in kilograms as ordinates. In the resulting graphs of each subject a monthly rhythm was unmistakably apparent in only a few of the cases. In the others, if periodic variations existed, they were masked by the unavoidable daily fluctuations. These daily chance variations were often of considerable magnitude, but could not be avoided because they depended upon the daily activities of the subjects, and it was impossible to obtain subjects whose activities could be controlled.

Most of the daily fluctuations could be accounted for by the records of the amount of sleep, physical and psychic disturbances, general health, and by the routine of the previous day. Loss of sleep and

TABLE 1

SUBJECT	PERIOD DURING WHICH TESTS WERE GIVEN	NUMBER OF CYCLES	NUMBER OF DAILY TESTS
1	November, 1920–June, 1921	8	134
2	January–March, 1921	2	27
3	January–March, 1921 September, 1921–April, 1922	7	119
4	November, 1920–April, 1921	5	79
5	February–April, 1921	2	45
6	November, 1920–June, 1921 October, 1921–April, 1922	*12*	*205*
7	November, 1920–April, 1921	5	77
8	January–March, 1921	3	36
9	November, 1920–June, 1921	5	108
10	January–April, 1921	3	56
11	January–March, 1921	3	42
12	October, 1920–April, 1921 November, 1921–April, 1922	*12*	*210*
13	January–March, 1921	2	37
14	January–March, 1921	2	45
15	February–March, 1921	2	28
16	November, 1920–June, 1921	8	145
17	January–April, 1921	3	57
18	February–March, 1921	2	25
19	February–April, 1922	2	30
20	January–April, 1921	3	64
21	January, 1921–February, 1921 September–November, 1922		58
22	March, 1921	1	18
23	January, 1921	1	18
24	November–December, 1920	1	20
25	January–February, 1921	1	13
26	February–March, 1921	1	13
27	January–February, 1921	1	19
28	November, 1920	0.5	12
29	March, 1921	1	10
30	January–February, 1921	1	18
Total 30	November, 1920–April, 1922	99.5	1767

physical discomfort tend to lower the strength, while pyschic disturbances, especially conditions of extreme nervous tension, have the opposite effect.

It is quite apparent that these daily chance variations might easily obscure periodically recurring changes in strength unless some means were taken to lessen their significance in the graphs. In order to do this a composite curve was made for each individual by superimposing the data for each monthly cycle. This was done by arranging the data for each cycle in successive rows, thus placing the data of corresponding days of the cycle in vertical columns. Thirty days was taken as the average length of the cycles, those which were more or less than 30 being arbitrarily shortened or lengthened in the intermenstrual period so as to keep the days of the menstrual period in corresponding columns. An average was obtained for each day of the monthly cycle, and these averages were used to plot the composite curve.

The averaged values for the monthly cycle should bring to light periodically recurring variations because these variations are constant for each cycle and are emphasized by data extending over a period of several months, while the daily variations are scattered indiscriminately throughout the different cycles and, therefore, are lessened in significance when a large number of cycles is averaged. According to the theory of errors, the influence of chance variations is inversely proportional to the square root of the number added; thus, if there are four cycles, the influence will be one-half as great as for one.

Composite curves plotted from the averaged values showed in practically every case periodically recurring variations. But the curves were still sufficiently irregular to warrant further smoothing. This was accomplished by a common statistical method; instead of using the averaged daily values, new values were obtained by averaging each with the values of the two adjacent days. This decreases extreme daily differences and brings out more clearly fundamental changes throughout the cycle.

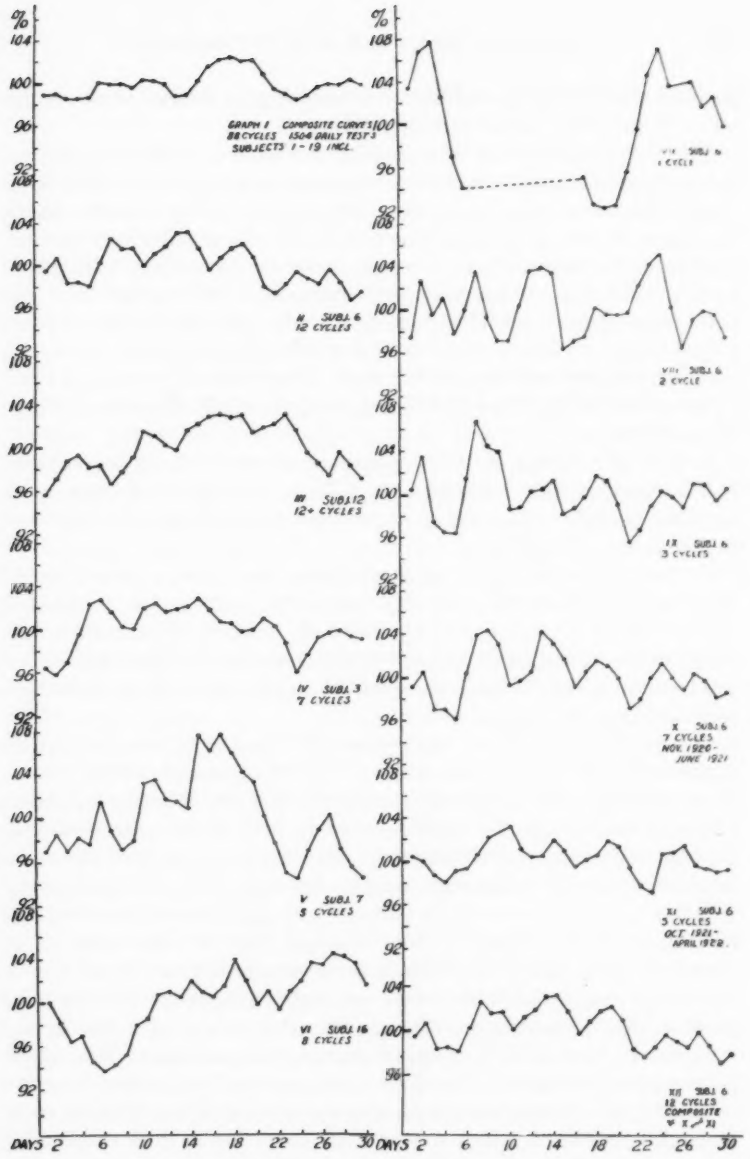
The smoothed averaged (composite) values for each day of the cycle were then reduced to percentage values for a more exact comparison of the individual records. These percentage values for each subject are given in table 2. Graphs of subjects 6, 12, 3, 7 and 16 plotted from these values are used as specimen curves (graphs 2 to 6).

Subjects 22 to 30 are not used in our conclusions because their tests extended throughout less than two complete cycles. Their data are considered valueless on account of the daily variations which cannot be diminished by averaging and the variations due to practice at the beginning of the experiment. The latter variation is not significant in the longer series. The records of these subjects are given, for the

TABLE 2

Percentage variations in the composite data for each subject with 100 as the average. Days of the monthly cycle begin with the first day of the menstrual period

SUBJECTS	1 DAY	2 DAYS	3 DAYS	4 DAYS	5 DAYS	6 DAYS	7 DAYS	8 DAYS	9 DAYS	10 DAYS	11 DAYS	12 DAYS	13 DAYS	14 DAYS	15 DAYS	16 DAYS	17 DAYS	18 DAYS	19 DAYS	20 DAYS	21 DAYS	22 DAYS	23 DAYS	24 DAYS	25 DAYS	26 DAYS	27 DAYS	28 DAYS	29 DAYS	30 DAYS
1	101.6	101.2	99.6	100.2	100.6	101.9	100.4	101.0	100.4	100.0	100.3	95.7	96.8	95.7	98.3	97.1	97.0	99.3	101.2	102.0	103.2	100.9	100.1	97.8	99.3	100.6	100.6	102.7	102.1	103.2
2	102.7	102.3	100.0	100.5	100.5	102.9	103.3			103.6	103.1	98.2	95.0	93.3				95.4	98.0	100.3	98.8						99.5	99.6	100.8	102.7
3	96.4	95.8	96.9	99.5	102.3	102.7	101.6	100.2	100.0	101.9	102.3	101.5	101.8	102.0	102.8	101.7	100.6	100.5	99.7	99.9	100.9	100.2	98.6	95.9	97.5	99.1	99.6	99.8	99.2	98.9
4	100.9	100.9	100.5	95.8	92.4	95.1	98.9	98.0	98.0	95.1	96.8	92.9	93.8	99.4	107.1	109.4	107.2	102.1	103.5	109.7	108.9	109.7	100.6	101.3	91.4	96.0	95.4	101.2	98.4	100.5
5	102.3	100.8	97.7	96.6	101.2	101.3	99.3	99.4	97.4	103.1	101.5	103.4	101.3	96.5	96.2	94.9	101.4	97.6	95.4	90.4	87.7	90.1	97.5	100.4	107.2	111.1	112.4		109.5	106.4
6	99.5	100.7	98.4	98.5	98.1	100.3	102.6	101.6	101.7	100.1	101.2	101.9	103.1	103.2	101.7	99.7	100.8	101.1	101.6	100.3	98.2	97.3	98.0	99.5	99.2	98.8	99.7	98.5	96.9	97.7
7	97.0	98.5	97.0	98.2	97.6	101.5	98.9	97.1	97.9	103.2	103.5	101.7	101.5	100.9	107.6	106.2	107.7	106.0	104.2	103.3	100.1	97.6	94.9	94.4	96.9	98.8	100.2	97.0	95.4	94.4
8	96.3	96.5	94.4	93.1	95.6	99.7	103.1	105.4	103.7	104.4	101.7	102.3	98.9	98.8	96.6	98.0	97.9	104.4	109.1	105.9	108.6	100.3	98.4	93.4	96.7	97.7	97.7	98.5		
9	96.1	94.6	96.7	99.8	102.3	104.9	104.9	107.0	105.4	103.6	99.7	98.1	97.6	97.1	98.5	97.7	100.7	105.4	106.4	104.8	99.2	99.8	98.3	97.1	95.2	97.9	98.9	98.5	97.3	95.5
10	102.2	100.9	98.9	98.8	98.6	98.3	97.5	97.4	101.3	100.8	99.3	101.4	99.1	101.3	98.6	100.2			98.0	97.3	97.7	103.8	100.3	101.0	100.4	101.5	99.3	101.3	101.9	103.0
11	94.8	95.2	100.6	104.4	104.1	100.8	103.7	106.3	105.2			104.7	101.7	100.8	98.6	101.5	105.9			103.6	102.0	95.3	96.5	94.0	98.0		96.4	97.7	93.2	96.1
12	95.7	97.1	98.8	99.3	98.2	98.4	96.7	97.8	99.1	101.5	101.1	100.2	99.8	101.6	102.1	102.9	103.0	102.8	103.0	101.4	101.9	102.2	103.1	102.7	99.6	98.6	97.5	99.6	98.4	97.2
13	91.5	98.2	101.6			100.4	98.7	98.0	104.0	107.9	109.8	105.4	110.3		111.6	113.1	105.1	99.1	97.2	97.2	97.3	92.2	89.0	89.9	92.3	93.0	94.7	101.4	100.8	95.8
14	103.2	106.2	106.3	105.9	101.0	99.4	98.8	99.3	95.1	92.3	94.1	97.7	101.7	104.0	105.3	106.7	106.4	113.0	110.9	114.0	107.9	100.2	93.8	91.6	96.5		94.5	96.0	97.2	101.2
15	102.5	96.7	96.5	96.8	99.2	98.6	99.9	99.7		99.7	97.5		96.7	98.9		99.1		101.3	98.9				103.9		99.3		103.9		103.7	107.7
16	100.0	98.1	96.4	96.9	94.5	93.7	94.2	95.1	97.9	98.4	100.6	100.9	100.4	101.9	100.8	100.4	101.4	103.8	101.9	99.6	100.9	99.2	100.9	101.8	103.5	103.3	104.3	104.0	103.4	101.4
17	97.6	97.6	97.7	95.2	93.9	99.7	100.5	99.1	92.1	94.9	96.5	96.5	95.9	95.9		99.1	98.8	103.1	101.7	103.2	101.6	105.7	106.4	105.4	100.5	96.6	107.0	107.3	110.2	99.7
18		100.0	98.0	98.7	99.1	101.4				102.2	101.3	97.9	95.0	93.6	93.2				101.6	101.8	102.0	98.8	102.3	105.6	107.6	102.6	98.9	98.7		99.8
19	100.4	98.8	97.7	96.8	98.2		97.1	97.5	96.3	96.1	96.1						100.2	105.6	106.8	106.8			103.4	103.9	100.9	101.2	100.8			
20	100.1	100.3	101.4	100.6	100.4	100.8	101.4	102.2	102.0	102.0	101.2	101.2	100.3	99.9	99.8	101.5	100.6	99.2	97.3	98.1	98.9	100.1	100.2	100.8	101.6	100.4	100.4	98.5	99.2	98.5
21	100.0	99.5	101.5	102.9	103.1	102.0	101.8	102.5	99.2	99.5	98.5	100.1	99.4	99.6	99.3	97.8	98.8	99.3	101.3	100.9	101.6	99.8	99.1	98.2	98.0	98.3	98.3	99.1	99.5	
22		102.9	102.1	100.2			98.4	98.1		97.9	98.2			98.4		99.1	99.3	101.7		102.8			102.5	102.5	101.0	98.3	96.3			99.1
23	111.1			107.8	104.0		96.8	87.2		86.2		85.8		83.6	88.2			91.5		98.3		106.8		113.2			116.3		111.7	111.5
24	108.7	109.8	108.6	103.2	101.2	99.1								91.2			91.1	87.6	92.4	94.8	97.1		100.0	100.6	109.3	96.5	98.7	104.1	105.6	109.2
25				97.9		97.2	95.1	98.1					101.3		108.8					106.6	105.5	95.9	97.0				97.4		100.6	99.4
26	100.0		104.3	109.3	105.5	98.6		97.4	98.5		95.7		95.1								95.1								100.4	100.4
27	101.3	103.6	107.8			108.5	101.1	97.9	101.9	102.2	105.5		101.7	101.9	97.3	96.7		100.6		101.4	101.3	94.2		101.3					108.0		
28	105.7	105.1															90.8	91.4	97.2	97.9	103.4	99.0		105.4	98.0	103.9		101.4		
29				102.3			103.2						98.9						94.8	95.9		99.3		101.1	100.6	101.8	102.4				
30		107.6	106.7				105.0	103.2		99.2		90.8	90.0	93.1			98.1	96.6	96.4	97.3			97.1	96.6	97.4	100.1	102.6			108.2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GRAPH I COMPOSITE CURVE OF 88 CYCLES 1504 DAILY TESTS SUBJECTS 1-19 INCL.
II SUBJ 6 12 CYCLES
III SUBJ 12 12+ CYCLES
IV SUBJ 3 7 CYCLES
V SUBJ 7 5 CYCLES
VI SUBJ 16 8 CYCLES
VII SUBJ 6 1 CYCLE
VIII SUBJ 6 2 CYCLE
IX SUBJ 6 3 CYCLES
X SUBJ 6 7 CYCLES NOV. 1920 - JUNE 1921
XI SUBJ 6 5 CYCLES OCT. 1921 - APRIL 1922
XII SUBJ 6 12 CYCLES COMPOSITE V X & XI
DAYS 2 6 10 14 18 22 26 30

Composite graphs of muscular efficiency variations. Ordinates, day of the monthly cycle: day 1, the first day of the menstrual period. Abscissae, percentage variations with 100 as the average.

purpose of showing the futility of attempting to determine the occurrence of a rhythm under such conditions.

The curves made from a composite of even two or three cycles are more irregular and the variations are of greater magnitude than those from a larger number of cycles. Apparently, within certain limits, the larger the number of cycles added, the more valid the rhythm. This is shown especially by a series of curves for subject 6 (graphs 7 to 12). This subject has very large and erratic daily variations. She is so unaccountably variable, in fact, that she was retained as a subject only because of her willingness and availability. One or even two months' data are valueless in her case. However, an average of three cycles reveals a rhythm which is progressively clarified by the addition of more cycles.

Subject 21 is about 40 years of age and, because of a hysterectomy, has no menstruation. Subject 20 is between 45 and 50 years, with irregular periods. These two are considered special subjects somewhat in the nature of controls.

Subjects 1 to 19, inclusive, were tested throughout from 2 to 12 cycles and are, therefore, the only ones considered in our conclusions. Practically all show a similar rhythm with definitely outlined features which seem to be typical and which are characteristic also of the composite curve (graph 1) made from the averaged data of these 19 subjects with a total of 88 cycles.

It is significant that the two curves with the largest amount of data (graphs 2 and 3) are more nearly identical than any of the others. It would seem that composite curves of data for these two subjects collected during a period from October to May of one year and from September to May of the following year, including totals of 12 cycles, would necessarily represent largely fundamental variations only. The fact that the curves of the two subjects agree adds strong evidence to this conclusion. So also does the fact that the one other curve (graph 4) most closely resembling these two is that of subject 3, who has a very stable disposition with noticeably few daily variations and these of slight magnitude. In general, the curves with the largest number of cycles have the greatest degree of resemblance. The atypical curves and those with the most variations are from scanty data, two or three cycles, within some cases a space of several days without data. It is also noteworthy that a composite curve for subject 6 including 7 cycles from November, 1920 to June, 1921 (graph 10) is practically identical with her composite curve for a similar period the following

year. The rhythm must be real and must be typical to be evidenced in two long series of tests.

The composite curve and the typical individual curves show a rhythm whose general outlines are represented by a menstrual decrease and an intermenstrual and premenstrual increase in muscular efficiency; that is, the strength is below average during the menstrual period and above average throughout the intermenstrual and premenstrual periods, which constitute about two-thirds of the monthly cycle. The menstrual drop maintains a sustained low level which may begin before the onset of menstruation and may continue until after menstruation. A second drop of less magnitude and of short duration, generally one day only, occurs about the 23rd day just preceding the premenstrual period. The strength during the premenstrual period may not again attain the high level of the preceding period or may be higher, but is always higher than that during menstruation. The intermenstrual increase is in general composed of three portions, a post-menstrual rise, a central high point, and a third peak just before the fall preceding the premenstrual period. Any one of these may be the highest. The individual curves vary somewhat as to the extent of these changes and their exact position in the curve.

The fundamental nature of this rhythm is further suggested by the fact that an astonishingly similar rhythm has been demonstrated by Moore and Cooper (4) in cardio-vascular changes and in respiratory rate. Subjects 2, 3, 6, 12, 16 and 19 were used in both tests. Individual peculiarities manifested in each subject are apparent in the muscular efficiency and cardio-vascular curves and respiratory rate; and must, therefore, be real and not artificial. Also the general character of the rhythm is the same in both series. It would seem that an underlying factor common to these physiological processes was responsible for the rhythm.

No doubt a composite curve of a much greater number of cycles would largely abolish the chance variations which necessarily occur in subjects whose activities cannot be entirely controlled. In the curves presented here there are unquestionably many such modifications which tend to obscure the monthly rhythm. All that we can hope to demonstrate is a general monthly periodicity somewhat similar in all cases but with minor configurations for which we are not able at present to account.

So little is definitely known as to what is actually occurring, what changes are taking place in the ovaries and uterus at each stage of the

monthly cycle, that it is at present impossible to account accurately for the phases of increased efficiency and the periods of depression. However, it seems reasonable to expect that, during the premenstrual period which according to one view, is the time of ovulation and uterine changes preparatory for the reception of the fertilized ovum, there would be a general increase in tone such that the general physiological condition would be improved. This would necessarily be followed by a reactionary period of depression coincident with the adjustments which take place and which are responsible for menstruation. Menstruation is apparently in any case a secondary, not a primary, event, and the depression occurring at that time is no greater than that occasioned by chance variations.

Menstruation is notably a phenomenon of the uterus, dependent upon periodic activities of the ovaries. It is merely one of the events in the monthly cycle and conceivably one of less significance than the ovarian changes which induce it. Subject 21 gives results illustrative of this point in that there is evidence of rhythmic changes even though no menstruation occurs. No attempt is made at present to account for the minor configurations of the rhythm, although they seem to be typical.

The whole question is being more fully investigated with the hope that more light may be thrown on the fundamentel phases occurring in the cycle and the alterations in bodily function accompanying these phenomena. We can, however, definitely conclude from the results so far obtained that there are periodically recurring variations in muscular efficiency and that the significant phase is the inter- and premenstrual rise and not the menstrual fall which has been over-emphasized by most workers, and that there is no indication of a marked depression during *normal* menstruation sufficient to interfere appreciably with women's activities.

SUMMARY

1. Daily observations of muscular efficiency in women over periods including from 2 to 12 monthly cycles each, with a total of 88 cycles, revealed periodically recurring variations coincident with the monthly cycle.

2. The typical variations are a menstrual decrease in strength followed by an inter- and premenstrual increase extending throughout the greater part of the cycle, but broken at about the 23rd day by a sharp fall of short duration.

3. There is no indication of a marked depression during *normal* menstruation sufficient to interfere appreciably with women's activities.

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MONTHLY VARIATIONS IN CARDIO-VASCULAR ACTIVITIES AND IN RESPIRATORY RATE IN WOMEN

LILLIAN M. MOORE AND CATHERINE R. COOPER

From the Department of Physiology, University of California, Berkeley

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Recent investigations on monthly periodicity in cardio-vascular activities and in respiratory rate in women have in general given negative results. The tendency at present seems to be away from the formerly accepted theory of a rhythm in these processes indicative of lowered activity during the menstrual period. The "wave theory" attained prominence through the work of Jacobji (1) as early as 1876, and it is only during the last decade that evidence has been obtained in apparent refutation. However, a careful review of the literature brings to light a decided paucity of data; that is, results from only a small number of subjects who were tested throughout, in most cases, one or two cycles only, with a subsequent inadequate analysis of the data.

It seemed worth while, therefore, to repeat and to extend the observations on these activities, especially since a two years' study of muscular efficiency in 30 women had revealed a typical monthly rhythm (2). If a similar rhythm in various cardio-vascular activities were demonstrated by a relatively few subjects, it should prove convincing.

Records were taken of the systolic and diastolic blood pressures and pulse rate by means of an Erlanger sphygmomanometer and the respiratory rate by means of a pneumograph. The auscultatory method was also used as a check. Pulse pressure was calculated and the product of this and the pulse rate was used as the circulatory index.

Eight subjects, students and faculty members of the University of California, were used. Six of these, nos. 2, 3, 6, 9, 12 and 16, were also subjects for the muscular efficiency tests, and the comparison thus made possible should give valuable results.

The investigation extended throughout practically the whole of the college year, from September 1921 to April 1922, inclusive, with a total of 609 determinations of blood pressure and 446 of respiratory rate during 41 monthly cycles. The records were taken on the average 4

and 6 days each week during the fall and spring semesters respectively. Subject 3 had an unbroken record for the 8 months. Subjects 12, 19 and 2 also had continuous records with the exception of a break during the Christmas holidays. Subject 16 was tested during the fall semester and subjects 6, 10 and 11 during the spring semester only. Four of the subjects had records including 7 monthly cycles each, an experimental period sufficiently long to establish uniform variations should they occur. The shortest records were for three complete cycles. Several other subjects were used for short periods but because their records included less than two cycles they were not considered in the final results. It seemed necessary to discard these latter because blood pressure and pulse rate are so variable and so easily affected by

TABLE 1

SUBJECT	PERIOD OF TESTS	NUMBER OF MONTHS	NUMBER OF CYCLES	NUMBER OF TESTS OF BLOOD PRESSURE AND PULSE RATE	NUMBER OF TESTS OF RESPIRATORY RATE
16	Sept. 15–Dec. 30	4	5	55	45
3	Sept. 15–Apr. 11	8	7	138	94
12	Sept. 15–Dec. 8, Jan. 16–Apr. 17	7½	7	108	74
19	Sept. 15–Dec. 9, Jan. 18–Apr. 15	7½	7	94	70
2	Jan. 16–Apr. 11, Sept. 16–Dec. 8	7½	7	80	59
6	Jan. 13–Apr. 5	4	3	43	32
10	Jan. 16–Mar. 25	3	3	44	33
11	Jan. 16–Mar. 25	3	2	47	39
Total of 8		44½	41	609	446

extraneous conditions that one or even two months' records are so complicated with fortuituous variations that any evidence of a rhythm is hopelessly obscured.

An effort was made to control the condition of the subjects by giving the daily tests at approximately the same hour for each individual after a uniform period of rest in a sitting posture. During the tests they were required to read something of a not too stimulating nature but interesting enough to hold the attention and keep the mental attitude more or less uniform.

Table 1 gives the number of subjects, periods tested; number of months, cycles, and daily tests each.

The results for systolic, diastolic and pulse pressures, pulse rate, respiratory rate and circulatory index for each subject were analyzed

Percentage variations in the composite data for each subject with 100 as the

	DAY CYCLE										
	1	2	3	4	5	6	7	8	9	10	11
Subject 6											
Per cent variation in:											
Systolic pressure	100.5	100.3	100.4	100.4	100.5	100.3	99.9	99.4		98.9	99
Diastolic pressure	98.6	98.9	100.2	101.9	103.4	103.9	102.8	100.6		98.6	98
Pulse pressure	104.2	103.2	101.3	98.7	96.7	95.9	96.7	98.4		100.1	101
Pulse rate	93.2	92.7	92.7	93.8	95.8	98.5	100.5	98.8		95.2	93
Index	96.5	95.0	93.3	92.2	92.5	94.5	96.9	96.8		94.7	92
Respiratory rate	102.0	99.6	98.2	97.5	96.4	96.1	95.5			97.8	100
Subject 12											
Systolic pressure	99.0	98.4	98.7	100.0	101.3	100.6	99.0	97.8	98.4	99.7	101
Diastolic pressure	98.9	97.8	97.8	98.9	100.5	99.9	98.4	96.8	97.9	99.9	101
Pulse pressure	100.0	100.0	100.8	101.5	102.5	102.5	101.5	100.8	100.0	100.0	101
Pulse rate	96.6	99.6	98.3	97.5	95.4	95.4	95.8	97.5	98.8	99.2	98
Index	98.9	99.0	98.7	98.6	97.4	97.1	96.6	97.5	98.3	98.7	100
Respiratory rate	99.3	100.0	99.3	98.7	98.0	98.0	97.3	96.3	97.5	95.7	97
Subject 3											
Systolic pressure	99.7	100.3	100.6	101.2	101.7	102.3	102.3	101.7	100.9	100.3	99
Diastolic pressure	103.9	102.1	102.5	101.7	100.8	100.0	100.0	100.0	100.0	99.6	99
Pulse pressure	98.2	97.3	97.3	100.0	103.6	108.1	108.1	106.3	103.6	102.7	101
Pulse rate	102.1	102.9	102.1	101.9	101.4	101.4	100.5	100.0	99.5	101.0	101
Index	98.3	96.3	95.4	97.8	101.3	105.5	105.8	104.5	101.7	100.5	99
Respiratory rate	102.3	103.0	102.3	101.7	101.0	101.0	100.3	100.0	99.7	101.0	101
Subject 16											
Systolic pressure	97.7	99.0	97.4	97.4	97.4	98.0	98.3	99.3	100.6	101.6	
Diastolic pressure	102.0	101.5	100.5	99.9	98.4	96.8	97.9	99.9	99.5	101.0	
Pulse pressure	94.2	93.3	90.8	93.3	95.8	98.3	99.2	98.3	100.0	100.0	
Pulse rate	94.6	97.0	99.8	100.7	101.2	101.6	104.0	104.9	104.9	102.6	
Index	88.0	89.2	89.5	93.5	96.7	100.1	102.7	102.6	103.9	101.7	
Respiratory rate	98.7	102.0		104.0	106.0	105.3	104.7	101.3	99.3	98.0	
Subject 19											
Systolic pressure	104.0	103.0	102.1	98.7	99.0	99.4	100.9	100.9	101.2	100.3	100
Diastolic pressure	102.8	101.4	100.0	100.0	100.0	100.5	102.3	103.3	103.7	101.4	100
Pulse pressure	98.2	97.3	97.3	96.4	97.3	97.3	98.2	97.3	97.3	98.2	98
Pulse rate	98.3	97.7	99.5	101.3	101.8	100.0	97.3	96.4	97.3		100
Index	96.6	97.3	98.4	96.4	95.4	94.7	95.1	94.7	94.6	96.7	96
Respiratory rate	102.2	102.2	105.2	104.2	100.2	93.8	94.3	94.3	98.7	93.0	97
Subject 2											
Systolic pressure	101.4	100.9	99.8	99.2	98.9	98.3	98.0	98.9	100.0		100
Diastolic pressure	103.8	102.9	100.0	96.7	95.7	95.7	97.1	98.6	99.1		98
Pulse pressure	98.7	98.0	99.3	102.7	103.3	102.7	100.0	98.7	100.0		102
Pulse rate	97.7	97.7	99.5	101.3	101.8	100.0	97.3	96.4	97.3		100
Index	96.3	96.1	99.0	103.9	104.8	101.9	96.8	95.9	98.2		103
Respiratory rate	96.7	96.0	98.7	102.7	103.3	102.7	100.0	99.3			96
Subject 10											
Systolic pressure	104.2	104.2	102.7	100.9	100.6	100.9	101.2	100.9	101.2	101.6	101
Diastolic pressure	106.5	106.5	103.3	100.5	100.5	101.9	102.8	101.9	100.9	99.1	99
Pulse pressure	97.6	100.8	102.4	101.6	100.8	100.0	99.2	100.0	101.6	104.9	105
Pulse rate	104.1	103.1	101.5	99.5	101.0	101.5	103.6	103.6	103.6	101.5	101
Index	101.2	102.8	103.3	101.1	102.5	101.7	102.9	102.8	104.0	102.0	106
Respiratory rate											
Subject 11											
Systolic pressure	101.3	101.0	100.1	99.3	98.7	99.3	99.8	100.4	99.8	99.3	
Diastolic pressure	102.4	102.8	101.9	100.9	100.0	100.4	100.9	101.7	100.9	100.0	
Pulse pressure	98.8	97.2	96.4	96.4	97.3	98.0	98.8	98.0	98.8	99.6	
Pulse rate	97.0	95.2	99.0	100.5	97.5	94.9	94.4	99.5	104.5	106.0	
Index	95.6	95.1	94.6	96.1	93.8	91.8	92.1	96.3	101.9	103.9	
Respiratory rate	101.4	100.0	100.0	100.0	100.7	102.8	104.2	108.4	108.4	109.8	

the monthly cycle begin with the first day of the menstrual period

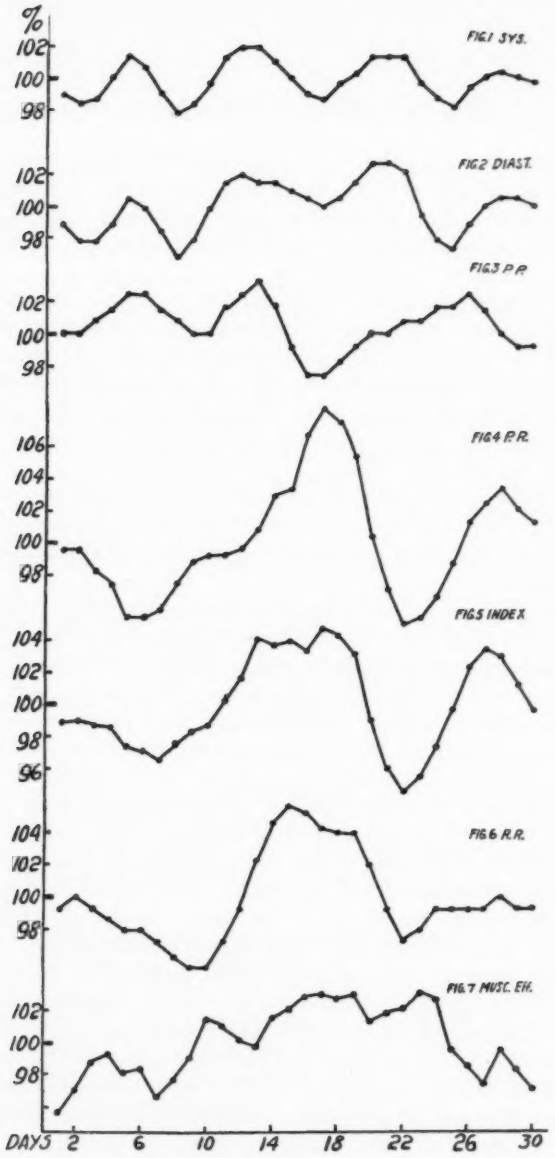
14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
99 9			99.3	98.7	98.5	98 9	99.3	99 3	99.1	99.1	99.7	100 8	101 7	102 1	101 7	101 0
99 8			98.6	96.7	96 0	96.3	97.4	98 6	100 3	101 5	102 6	102 8	103 0	102 2	100 9	99.4
100.1			100.4	101.2	101.9	102.5	102 3	100 4	97.2	95.0	95 0	97.4	99 7	101 9	103 2	104.1
102 1			104.3	107.1	107.4	108 4	107.5	105.7	104.6	103.9	103.7	103.7	102.1	100.7	98 0	95.8
102 3			108.2	110 8	111.8	110 5	109.8	103.1	102.1	98.3	97.3	99 2	100 9	102 7	101 4	99 6
					107.8	105 8	103.2	101 2	100 0	98 9	97.5	96 6	98 0	100 7	103 6	103 6
101.0	100.0	99 0	98.7	99.7	100.3	101.3	101.3	101.3	99.7	98 7	98 1	99 4	100.0	100 3	100 0	99 7
101.5	101.0	100 5	100 0	100.5	101.5	102 6	102.6	102.1	99 4	97 9	97.3	98 9	100 0	100 5	100 5	100 0
100.8	99.2	97.5	97.5	98.3	99.2	100 0	100.0	100 8	100.8	101.7	101.7	102 5	101.5	100 0	99 2	99 2
102 9	103.3	106.7	108.3	107.5	105.4	100.4	97.1	95 0	95.4	96.7	98.7	101 3	102.5	103 4	102 1	101 3
103.7	103.9	103.3	104.7	104.3	103.1	99 0	96 0	94 6	95 5	97.4	99.7	102 3	103 4	103 0	101 2	99 7
104 7	105.7	105.3	104.3	104.0	104.0	102.0	99.3	97.3	98.0	99.3	99.3	99.3	99.3	100 0	99.3	99.3
98.8	99.4	99.4	99.4	99.1	99.1	99 4	99.4	99.4	99.1	99.7	100 6	101.2	100 9	100 3	99.7	99 7
99.6	101.3	101.3	100.4	99 6	99.6	100 0	99 2	97.9	96.6	97.5	99 6	101.3	101 7	100 4	100 0	100 0
98.2	96.4	96.4	97.3	98.2	98.2	98 2	100.0	102.7	104.5	104.5	102.7	100 9	100 0	100 0	100 0	99 1
95.6	95.1	98.5	100.5	100 5	99 0	98 1	100 5	101.4	101.9	100.0	99.5	103 0	102 9	104 3	103.3	102 9
96.8	96 0	96.3	96.8	96 9	95 9	95 3	96.7	99 8	103 3	105.9	105 6	104.2	101 9	102 6	102 3	101.6
96 0	95 3	98.7	100 3	100 3	99 0	98 3	100.7	101 3	102.7	100 0	99 7	101.0	102.7	104 0	103 0	102 7
99.3	99.7	100.3	100.3	101 0	101 0	101.3	101.3	102 2	102.5	101 9	100 6	100 6	101.3	101 3	100.8	100.3
97.4	98.0	99.0	99.0	101.0	101.0	101.8	99 5	100.5	101.0	101 0	99.0	98.0	98 0	98.5	99.5	100.5
100.0	100.0	100.0	100.0	98.3	98 3	98.3	101.7	102 5	102 5	100.8	100.8	102.5	104.2	103.3	100 0	97 5
104.4	103.5	103.5	103.5	102 6	102 6	102 6	104.4	103 5	101 2	98.8	97.9	99 8	101.2	102 1	98 8	96 0
103 5	102.7	102.6	102.7	100.3	100.6	101.0	105.7	105.3	102.7	98 9	97.4	100.8	104.0	104.6	98.4	92.8
92.7	95.2	96.7	98.7	96 0	94.0		92.7	95 3	99.3	101.3	99.3	96.7	96.7	98 7	98.7	98 0
99.0	99.0	98.7	97.8	97 5	98 4	100 6	102.5	103.1	101.2	99.1	98.1	98.7	99.7	100 6	101 3	101 9
98.1	98.1	99.1	98.6	98.1	97.7	100.0	102 8	104.2	102 3	100.0	99.1	98.6	98.1	97.7	100 0	102 3
100 9	100.0	97.3	94.7	95 6	100 0	103 5	103.5	101.8	98.2	96.5	95.6	99.1	102.6	106.2	104 4	101 7
101.3	104 3	105.2	103.5	102 6	102.6	103.0	103.5	103.5	103.5	102.2	100.4	100 0	100.9	101.7	100.4	98 3
104.1	105.0	102.5	98.7	99 0	103.4	106 8	106.8	105.3	102 4	99.3	96.7	99.4	103 8	108.1	104.8	100 0
103.5	100.2	98.5	98.0	98 0	94.7	94 7	94.7	98 0	98 5	100 0	100 0	101 5	103.3	106.3	107 0	104 7
98.6	98.0	98.0	98.9	100.8	102.5	102.5	100.8	98.9	98 0	98.9	100 8	102.5	102.8	103 0	101.4	101.7
97.1	97.1	97.1	98.1	102.1	106 2	107.6	104.8	101 4	99.1	99.1	100.0	100.9	100.5	99.5	99 5	101 9
100 0	99.3	99.3	100.0	99.3	98.7	96.7	95.3	94.0	95.3	98.0	102.0	104.7	106.0	105.3	104 0	101 3
101.3	99.1	100.4	103.1	107.6	107.6	104.9	100 0	97.8	96.9	98.2	99.1	99.5	97.7	97.3	97.3	97.3
101.1	98.5	99.6	102.4	105.0	104.1	99.9	94.1	90.7	91.3	95.4	101.0	104.5	104.0	101.7	100 1	98 9
103.3	102.0	100.7	98 0	99.3	101.3	102.7	102.0	100.7	100.7	102.0	102.7	102 0	100.7	100.0	100 0	98.7
99.7	98.8	97.6	97.6	97.9	99.4	100.3	100.3	99.4	98.2	97.6	97.6	97.9	99.1	99.7	101.2	102.4
100.5	99.5	97.7	97.7	98.1	100.5	101 9	100.9	99.1	96.3	96.3	96.3	97.2	97.2	97.7	99.5	102 9
99.2	97.6	97.6	97.6	97.6	97.6	97.6	99.2	100 8	102.4	100.8	100 8	100 8	100.8	97.6	94.3	94.3
99.5	97.4	98.4	100.5	104.1	104.6	103.1	99.0	96.4	96.6	94.9	95.4	96.4	99.0	99.0	99.5	101.0
98.0	94.8	95.6	97.8	101.5	102.1	100 3	97.7	96.6	97.5	95.1	99.1	97.2	99.8	97.2	94.8	99.0
	99.6	99.9	100.2	98.5	97.9	97.6	99.3	100.4	101.0	100.7	100.7	100.7	100.7	100 4	100.7	101.0
	98.3	97.9	99.1	97.9	98.3	97.9	99.1	99.6	100.0	100.0	100.4	100.4	100.4	99 6	100 0	100 9
	102.8	103.6	102.8	100.4	98.0	97.2	99 6	102 0	102.8	102.0	101.2	102.0	102.0	102.8	102.0	101 2
	96.5	96.5	99.5	102.5	100 5	101.5	105.0	108.5	107.0	101.5	99.0	100.5	101.5	102.0	99.5	99.0
	98.7	99.8	101.4	101.7	97.5	98.3	104.0	110.1	109.8	103.9	100.6	102.3	103.0	104.3	101.4	100.3
	100.7	97.9	100.7	97.9	95.2	90.3	91.0	91.7		92.4	94.1		97.9	105.6	107.0	105.6

separately. First the average length of the cycles was determined and found to be 30 days. A composite of the data for the 30 days of each cycle was made to diminish the significance of chance variations. Before plotting curves, these values were smoothed by averaging the data of each with those of the two adjacent days. The smoothed averaged (composite) values for each day of the cycle were then reduced to percentage values for a more exact comparison of the various curves. This latter step in the analysis is of utmost importance since the actual quantitative variations in the units of measurement used are of such different orders of magnitude. The percentage values for each subject are given in table 2. The graphs of subject 12, figures 1 to 6, are used as specimen curves. A graph (fig. 7) of the muscular efficiency variations of the same subject, a composite of 12 cycles is given for comparison.

The graphs of the activities tested indicate a typical rhythm comparable to that previously demonstrated for muscular efficiency: that is, one with a low menstrual and high intermenstrual phase. The menstrual period is uniformly low in all cases and is generally followed by a slight post-menstrual rise which in turn precedes a post-menstrual fall on about the 8th day. The highest sustained level in the curve is in the intermenstrual period from the 14th to the 18th day. This is especially pronounced in the curves of the circulatory index and respiratory and pulse rate. The premenstrual period as a whole is, in most cases, lower than the intermenstrual even though the 30th or last day may be the highest in the cycle. (The high record of the last day is obscured by averaging with the low record of the first day of the following cycle, and therefore is not apparent in the graphs.) There is a very characteristic drop, often of considerable magnitude but of short duration, just preceding the premenstrual rise.

In the original data for each cycle there are many variations in the intermenstrual period which are apparently of a fortuituous nature. Drops occur but there is never a sustained low portion such as that coincident with the menstrual period. The greater the number of cycles averaged to obtain the final results, the more these intermenstrual chance high and low portions are leveled and diminished in significance while more data increase the prominence of the typical variations. The latter would seem, therefore, to be due to periodic changes.

No doubt a composite curve of a much greater number of cycles would largely abolish the chance variations which necessarily occur in subjects whose activities cannot be entirely controlled. In the



Explanation of graph: Subject 12.

Ordinates, Days of the monthly cycle; day 1, the first day of the menstrual period.

Abscissae, Percentage variations with 100 as the average.

Figs. 1–6. Composite curves of 7 cycles: Sept. 1921–April 1922.[1]

Fig. 7. Composite curve of 12 cycles. Oct. 1920–April 1921, Nov. 1921–April 1922.

Sys., systolic blood pressure; *Diast.*, diastolic blood pressure; *P.P.*, pulse pressure; *P.R.*, pulse rate; *Index*, circulatory index; *R.R.*, respiratory rate; *Musc. eff.*, muscular efficiency.

curves presented here, there are unquestionably many such modifications which tend to obscure the monthly rhythm. All that we can hope to demonstrate is a general monthly periodicity somewhat similar in all cases but with minor configurations for which we are not able at present to account.

The fundamental nature of the rhythm is further indicated by the similarity between the various cardio-vascular and respiratory curves, especially those of pulse rate and respiratory rate, and between these and the muscular efficiency curves (2). If the rhythm were due merely to chance variations, there would be small probability that the same typical picture would be presented by blood pressure, respiratory rate and muscular efficiency changes.

There is not only a typical rhythm common in general to the activities studied but there are also characteristic variations for each individual evidenced in each of the curves and varying somewhat from those of the other subjects.

Subject 12, (figs. 1 to 6) who was observed throughout seven cycles presents what might be considered a more typical curve than any of the others, perhaps because she led a rather uniform existence and was very accustomed to the procedure of the tests. Her curves show, however, certain uniform individual variations such as a pronounced menstrual and post-menstrual fall and a very marked depression just before the premenstrual rise corresponding to those in her muscular efficiency (fig. 7). Subject 16, tested for five cycles shows the same peculiarity apparent in her muscular efficiency (2), that is, a sudden extensive menstrual drop beginning before the onset of menstruation, followed by a sudden post-menstrual rise which is sustained or gradually increased during the remainder of the cycle. Subject 3, tested during seven cycles has a characteristic marked post-menstrual rise. Subject 2, also tested for seven cycles was extremely variable but gives, nevertheless, a rather typical rhythm with a peculiar premenstrual pseudo-plateau. Subject 19, seven cycles, who is also variable, has prolonged low menstrual and post-menstrual periods comparable to those in her muscular efficiency. Subjects 10 and 11 were tested for two or three monthly cycles and at irregular intervals. They give merely indications of a general rhythm largely obscured by the chance variations which could not be eliminated because of the paucity of the data for each day.

The occurrence of peculiar individual variations which are evidenced generally in each activity studied would seem to indicate a common cause. The similarity is especially marked in the cases that are pre-

sented by a composite of a large number of cycles, as in subject 12, who also has a long muscular efficiency record. This subject has few daily fluctuations and these of slight extent so that her periodically recurring changes are clearly demonstrated. The parallelism between respiration and pulse rate in most cases is especially striking.

Four smaller rhythms are also suggested in many of the curves. These are variable but rather persistently evident. They are necessarily not calendar weekly rhythms since the cycle is 30 days in length and the days of the week would fall in different phases of each cycle.

The observations so far indicate an unmistakable rhythm coincident with the monthly cycle, the lowest point occurring during the menstrual and the highest during the intermenstrual period. The low records of the menstrual period are not, however, of greater magnitude than many occurring as chance variations, and therefore do not signify an extensive general lowering of the physical condition sufficient to interfere with women's activities. They rather represent a phase in the rhythm accompanying the fundamental periodic changes which occur throughout the whole monthly cycle.

The tests are in part being extended in another series of determinations the results of which will be published later.

SUMMARY

1. Daily observations of cardio-vascular activities and respiration rate in women over periods including from 2 to 7 monthly cycles indicate periodically recurring variations coincident with the monthly cycle.

2. The typical variations are a low menstrual and a high intermenstrual period.

3. The rhythm is especially marked and uniform in the pulse rate, circulatory index and respiration rate.

4. The low records of the menstrual period are not greater than many occurring as chance variations, and therefore do not signify an extensive general lowering of the physical condition. They rather represent a phase in the rhythm accompanying the fundamental periodic changes which occur throughout the monthly cycle.

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STUDIES ON THE PATHOGENESIS OF TETANY[1]

I. The Control and Cure of Parathyroid Tetany by Diet

LESTER R. DRAGSTEDT and SILBER C. PEACOCK

From the Hull Physiological Laboratories of the University of Chicago

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Symptoms of tetany which closely resemble those observed in human pathology can be most easily produced in animals by the complete removal of the parathyroid glands. The pathogenesis of this form of experimental tetany and of parathyroprivic tetany in man is without doubt the same. The other forms of tetany seen in man, idiopathic or occupational tetany, infantile tetany or spasmophilia, gastro-intestinal tetany, the tetany of pregnancy and lactation, eclampsia, tetany in infectious diseases and following the administration of various poisons, have not been definitely related to the parathyroid glands. The chief difficulty has been the failure to find anatomic evidence of injury or degeneration in the glands in these various conditions.

It has been definitely established that the thyroid and parathyroid glands are anatomically and functionally distinct and separate organs, and that the acute nervous manifestations which follow upon the complete removal of these glands in carnivorous animals are due solely to the absence of the parathyroids. However the functions of the parathyroid glands and hence the pathogenesis of the symptom complex which results from their removal are still obscure. Their great importance is indicated by the fact that, under ordinary conditions of diet and care, the great majority of animals die very soon if the glands are removed. This fact has led to the common belief that they are not only necessary for the prevention of tetany but for life as well. The animals that occasionally survive the operation (5 to 6 per cent) are usually considered to have accessory parathyroid tissue in some different region. At present the most popular theory of their function is that of internal secretion. The glands furnish some substance to the

[1] A preliminary report of this work was published in the Journal of the American Medical Association, Nov. 4, 1922, lxxix, 1593.

blood, necessary for normal metabolism and in the absence of which the individual quickly dies with symptoms of tetany or severe depression. The early theory, and one which the present experiments strongly support, asserts that the glands function by the method of detoxication. Poisons in the blood stream are removed or destroyed by the parathyroid cell. It is of course possible, if not probable, that the glands may produce some hormone capable of neutralizing such toxic bodies or of stimulating some distant organ to such function.

The experimental results of several recent investigators make it practically certain that animals in tetany or depression following parathyroidectomy are suffering from a severe intoxication. Koch (1) recovered a number of toxic bases such as guanidine, methyl guanidine, histamine, choline and trimethylamine, from the urine of such animals, while Paton (2) and his associates found an increased amount of some of these toxic bodies in the blood. Vassale and Generali (3) early believed that the parathyroids had some antitoxic action against some products of metabolism, a view shared by a number of contemporary workers. Conclusive evidence has been obtained recently by Luckhardt (4) and his associates, in a series of experiments carried on independently and simultaneously with those here reported, that dogs in parathyroid tetany or depression are suffering from an intoxication. They find that the responsible toxic substances are water soluble, and may be removed from the blood by inducing a vigorous diuresis through the intravenous injection of large amounts of Ringer's solution.

Nervous manifestations which in many particulars are identical with those of parathyroid tetany have been observed in animals following various experimental procedures which do not directly involve injury to the parathyroid glands. We have occasionally noted tetany in dogs following the production of an acute obstruction in the duodenum, an occlusion of the pylorus, or the production of closed isolated intestinal loops. The feeding of excessive quantities of meat to Eck-fistula dogs or animals with extensive liver injury following phosphorus or chloroform poisoning often causes convulsions similar to those seen in parathyroid tetany. It is a common observation that the feeding of meat to para-thyroidectomized dogs accelerates the onset and increases the severity of the tetany, while conversely starvation or carbohydrate feeding delays the tetany and fatal issue.

Many of these observations are in harmony with the theory that tetany is an intoxication and that perhaps many forms of tetany have a pathogenesis much in common. While it is possible that the toxic

substances in parathyroid tetany arise in the body tissues through a defect in protein metabolism, the observations listed above point strongly to an exogenous origin, and particularly to the gastro-intestinal tract. Roger and Garnier (5) demonstrated that the normal contents of the stomach and intestines are toxic if injected parenterally in animals and these observations have been repeatedly confirmed. In previous studies by one of us (6) on the nature of the toxemia in intestinal obstruction, it was found that the responsible toxic substances are produced in the intestine by the activity of the proteolytic group of intestinal bacteria. They belong to the group of toxic amines, and histamine (7) has been isolated from the obstruction poisons. It has also been found in the normal intestine and feces (8). The toxicity of the intestinal content is almost entirely due to the products of bacterial proteolysis, chiefly toxic amines, and their production may be controlled to a large extent by factors which control the intestinal organisms, and their metabolism. A diet rich in proteins of animal origin leads to the development of a bacterial flora almost exclusively proteolytic in character and the consequent production of large amounts of toxic amines. On the other hand a carbohydrate diet, more particularly one containing definite amounts of either laxtose or dextrin, produces a change in bacterial types so that the intestinal flora becomes almost entirely fermentative or aciduric. Of perhaps greater importance is the fact pointed out by Kendall that the presence of utilizable carbohydrate produces a change in the metabolism of intestinal organisms, so that proteins in the medium are spared and the production of poisons prevented. We have found that the toxemia incident to intestinal obstruction may be delayed according to the degree to which this bacterial proteolysis can be checked (9). If the toxic substances responsible for parathyroprivic tetany come from the intestinal tract, then it would seem that the tetany might be delayed or prevented by dietary measures which prevent the formation of these poisons.

EXPERIMENTAL PROCEDURE. *Group I.* Experiments with young dogs (2 to 3 months old). The individual protocols are tabulated (table 1).

The six dogs in this group were given a diet of white bread and whole milk ad libitum and 60 grams of lactose per day. In from 4 to 6 days the feces became liquid, odorless, markedly acid to litmus, and smears of the fecal bacteria showed a marked predominance of Gram positive organisms having the morphology and arrangement of the aciduric type. As soon as this change occurred the thyroid and parathyroid glands were

removed. The animals quickly recovered from the operation and were continued on the special diet. Two died on the 4th and 5th day and the remaining four on the 9th and 10th day following the operation. None of them showed any symptoms of tetany at any time. All authors are agreed that the symptoms following parathyroid removal in young animals are more acute and death occurs much sooner than in adults. The usual survival is not more than 3 days.

TABLE 1

Group I. Experiments with young dogs (2 to 3 months old). Diet: White bread and whole milk ad lib., lactose 50 grams per 24 hours

NUMBER OF ANIMAL	DAYS ON DIET BEFORE OPERATION	FECES AT TIME OF OPERATION	DAYS SURVIVED THYROPARATHYROIDECTOMY	TETANY	DEPRESSION	REMARKS
1	4	Liquid Organisms Gram negative	4	None	None	Death occurred suddenly
2	4	Liquid Odorless	5	None	None	Refused food on 2nd day. Death sudden
3	7	Liquid Odorless	9	None	+ on 8th day	Refused food on 5th day. Death in depression
4	7	Liquid Odorless	9	None	+ on 8th day	Refused food on 7th day. Death in depression
5	7	Liquid Odorless	9	None	+ on 8th day	Refused food on 7th day. Death in depression
6	14	Liquid Odorless	10	None	None	Death occurred suddenly

Group II. Experiments with adult dogs (table 2). Eleven dogs in this group were fed a diet of boiled rice (500 grams), beef heart (100 grams) and lactose (50 grams) per day. According to Torrey (10), such a diet brings about a marked predominance of aciduric organisms and an almost complete suppression of the putrefactive type. It consists of approximately 172 grams of carbohydrate, 20 grams of fat and 30 grams of protein. The absence of milk from this diet is significant since it is

well known that calcium, which is present in relatively small amounts in milk, may in large doses relieve the early symptoms of parathyroid tetany. The lactose was given in solution by stomach tube once or twice daily. This diet was found to bring about the change in intes-

TABLE 2

Group II. Experiments with adult dogs (2 to 4 years old). Diet: Boiled rice 500 grams, beef heart 100 grams, lactose 50 grams, per 24 hours

NUMBER	DAYS ON DIET BEFORE OPERATION	FECES AT TIME OF OPERATION	DAYS SURVIVED OPERATION	TETANY	DEPRESSION	REMARKS
9	7	Odorless, bacteria Gram +	7 days	+ on 5th day	None	Distemper. Refused food on 5th day
10	7	Bacteria about 50% Gram +	1 year	+ on 4th day recovery	None	Still living
11	7	Bacteria about 50% Gram +	4 days	+ on 2nd day	None	Died in tetany
12	4 70	Bacteria about 70% Gram +	9 days	+ on 4th day recovery	None	Died from pneumonia
13	4	Bacteria about 70% Gram +	1 year	None	None	Still living
14	4	Bacteria about 50% Gram +	5 days	None	+	Died from pneumonia
15	4	Bacteria about 50% Gram +	1 year	None	None	Still living
16	4	Bacteria about 50% Gram +	1 year	+ on 4th day recovery	None	Still living
17	4	Bacteria about 50% Gram +	9 days	+ on 3rd day recovery	+	Died in depression
18	4	Bacteria 50% Gram +	9 days	+ on 2nd day	+	Died in depression
19	4	Bacteria 50% Gram +	7 days	+ on 2nd day	None	Died in tetany

tinal flora described by Torrey and the feces became odorless. They were not however liquid, nor were they acid in reaction. After a preliminary feeding of from 4 to 7 days, the thyroid and parathyroid glands were removed, and the diet continued as before. Eight of the dogs

showed symptoms of tetany on the 3rd to 5th day. Two died in tetany on the 3rd day, five died of either tetany or depression on the 8th or 9th day, and four of the animals or 36 per cent recovered from all tetany symptoms and are still alive (1 year).

Group III. Experiments with adult dogs (table 3). The third group of nineteen adult dogs was fed a diet of white bread and skimmed milk ad libitum, and lactose (50 to 125 grams per day). As the experiments progressed we became convinced that this diet was far more efficacious in checking bacterial proteolysis in the intestine than the other. The lactose was given in solution in the milk and the dogs soon learned to take it voluntarily. In most cases the effect on the feces was manifest at the end of a week and in all after 2 weeks of such feeding. The feces became odorless, liquid in character, light brown, acid to litmus, and the bacteria present were almost exclusively of the aciduric type, both in morphology and staining reactions. At varying intervals after this change had taken place, the thyroid and parathyroid glands were removed. Twelve of the animals showed transient symptoms of tetany on the 3rd or 4th day. Two died of pneumonia (distemper) on the 10th day. Three died in the 3rd week, and two in the 5th week in tetany or depression, and one in the 5th week from the severe enteritis produced by the diet. Eleven or 60 per cent recovered from all tetany symptoms and continued in good condition for many months. Several of these died in tetany after 4 to 9 months. The majority however are still living; in several cases more than a year since the operation. When it is recalled that the average survival of adult dogs following parathyroidectomy is not more than 4 or 5 days, it will be seen that all of these animals outlived the usual period. Of greater significance however is the fact that 60 per cent of them survived for many months and several have already lived a year. Of dogs without such treatment, about 4 or 5 per cent will survive the operation and continue in good condition thereafter. These usually show no post-operative tetany whatever and careful examination will show the presence of accessory parathyroid glands.

The four animals of the second group and the seven of the third group that recovered from the thyro-parathyroidectomy are in a good state of nutrition and are gaining in weight. There are no symptoms referable to thyroid insufficiency. In both groups the special diets were continued for 5 or 6 weeks after the operation. Attempts to substitute the usual stock diet or one of meat for the special diet previous to this time resulted in more or less severe tetany. However, after 6 weeks all of the animals could take the usual laboratory diet of meat and vege-

TABLE 3

Group III. Experiments with adult dogs (2 to 4 years old). Diet: White bread and whole milk ad lib., lactose 50 to 125 grams per 24 hours

NUMBER	DAYS ON DIET BEFORE OPERATION	LACTOSE PER 24 HOURS	FECES AT OPERATION	DAYS SURVIVING OPERATION	TETANY	DEPRESSION	REMARKS
		gm.					
20	10	50	Liquid	30	+ Recovery	+ Recovery	Died—enteritis
21	10	50	Liquid	11 months	None	None	Living
22	11	50	Liquid	9 months	+ Recovery	None	Died in tetany
23	7	50	Liquid	15 days	+ Recovery	+ Recovery	Died in depression. Ligated pan. duct
24	8	70	Liquid	16 days	+ Recovery	+ Recovery	Died in depression
25	8	50	Liquid	8 months	None	None	Living
26	7	50	Liquid	10 days	+	+	Acute int. obstruction
27	9	80	Liquid	4 months	+ Recovery	None	Died in tetany
28	18	80	Liquid	9 months	+ Recovery	None	Living
29	7	90	Liquid	4 days	+	+	Pregnant
30	5	70	Liquid	28 days	+ Recovery	None	Died in tetany
31	28	70	Liquid	6 months	+ Recovery	−	Living
34	21	30	Liquid	10 days	+	+	Died in depression
35	21	60	Liquid	43 days	+ Recovery	−	Died in tetany
36	6	100	Liquid	4 months	+ Recovery	−	Living (pregnant)
37	5	100	Liquid	4 days	+	−	Pregnant. Died in tetany
38	14	70	Liquid	4 months	+ Recovery	−	Living
39	21	50	Liquid	13 days	+	− (Very warm day)	Died in tetany
40	21	50	Liquid	4 months	+ Recovery	−	Living

tables without the production of any adverse symptoms. The mechanism of this readjustment of the body to the loss of the parathyroid glands is at present under investigation.

Discussion. The six animals in the first group survived the removal of the parathyroid glands for an average of 7.66 days. This is about three times the average survival of dogs at that age on ordinary diets and without other treatment. In the light of knowledge obtained from later experiments it is practically certain that another group of such young dogs could be kept alive much longer by more accurate dietary control. For a day or two following the operation there is usually a loss of appetite and many animals refuse food altogether. This is doubtless partly due to the trauma of the operation. Such animals gradually develop tetany or depression and the aversion for food becomes correspondingly greater. Material given by stomach tube is promptly vomited, the animal becomes progressively more toxic and finally dies in tetany. In such cases the daily administration of milk and lactose in solution by stomach tube beginning immediately after the operation will restore the animal to good condition and normal appetite.

The experiments with the second group bring out several important points. If the animals that died from pneumonia are excluded, it will be seen that 45 per cent of this series recovered from the parathyroidectomy and have remained alive for several months. Milk is therefore not a necessary determining factor in the diet, since these dogs received no milk. The diet contained from 2 to 3 grams of protein per kilogram of body weigth (calculated value), a relatively high protein intake. The rate of protein katabolism in the body was therefore not decreased by the special diet, an observation which indicates that the toxic factor is probably not an endogenous metabolic product.

The results obtained with the third group are perhaps still more conclusive. Of these nineteen animals, all but two survived the parathyroidectomy longer than dogs on ordinary diets (average 4 to 7 days). These two animals lived 4 days and died in tetany. Both however were pregnant and it is well known that this condition aggravates the symptoms and greatly hastens the fatal issue. Carlson (11) and Werelius (12) report that pregnant dogs die within 24 hours following parathyroid removal. Sixty-three per cent lived more than a month and 47 per cent more than 4 months following the operation. Seven, or 37 per cent, are still living (4 to 12 months since the operation).

There can be no question but that the diets described above greatly influence the tetany or depression which commonly follow parathyroid-

ectomy, and in many cases prevent it altogether, allowing the animal to survive in good condition for at least as long as a year. The large number of dogs operated upon precludes the possibility that the unusual survival is due to accessory parathyroid tissue. Marine (13) states that from 5 to 6 per cent of dogs show such accessory glands and in my experience, 96 to 97 per cent of dogs die in either tetany or depression following thyro-parathyroidectomy. Search for accessory glands was made in those animals that died at later periods but none were found. Little significance is however attached to this failure since apparently a few parathyroid cells can function for the whole and there is no reliable anatomical criterion for distinguishing them from other cells of similar structure. More reliance was placed on a physiological test. Parathyroidectomized animals that showed no evidence of tetany or depression whatever after 2 or 3 weeks were allowed to eat meat. The appearance of severe tetany within 48 hours was taken as an indication of absence of accessory parathyroid tissue. The effects of the diet are undoubtedly chiefly local, namely, in their influence on bacterial proteolysis in the intestine and the production of bacterial poisons. Starvation does not prevent the appearance of tetany nor do diets rich in other carbohydrates than lactose and dextrin. The latter are relatively slowly absorbed and appear in the feces when as much as 75 grams are given by mouth to a 10 to 15 kilogram dog. If the effect of the carbohydrate were systemic then other sugars such as cane sugar, dextrose, etc., should be equally or more efficient. Although the local effect of the diet is not certainly known, it seems most probable that the sequence of events is as follows. The lactose is present in all parts of the intestine and forms a readily available source of carbohydrate for the intestinal organisms. These break down the lactose, sparing the proteins, and the resultant products gradually increase the acidity of the mediums. Conditions become more and more unfavorable for the proteolytic bacteria, which grow best in an alkaline medium, and more favorable for the aciduric group, which grows best in a medium slightly acid. Thus after a week or two of lactose feeding, bacterial proteolysis in the intestine becomes practically checked, first through a change in metabolism and later through the resultant change in bacterial types. The aciduric group apparently can not break down proteins to any great extent. The rôle of the milk in the diet is not entirely clear. It has long been known that an exclusive milk diet will delay the onset of parathyroid tetany in dogs. It will not prevent it however and is not so effective as an equal volume of 30 per cent lactose solution in water.

Nevertheless the addition of milk to the diet seems to have a definite beneficial influence. The animals at no time were given more than one liter and usually less than one-half liter of milk per day. Thus the calcium present in the diet because of the milk never exceeded 1.6 grams per day, an amount which given by mouth has never been demonstrated to have any effect on tetany.

The fact that completely parathyroidectomized dogs, when kept alive for 5 or 6 weeks by dietary treatment, may continue in good condition thereafter without treatment raises the question of successful grafts. There are many cases in the literature where symptoms of tetany due to parathyroid insufficiency or possible absence have been apparently cured by parathyroid transplantation. Such cures cannot be interpreted to establish normal function of the transplant since these experiments indicate that some other readjustment possibly involving vicarious action of other tissues can account for the disappearance of symptoms.

It is much easier to prevent the appearance of tetany or depression in parathyroidectomized animals than it is to cure it once it has become established. The explanation is perhaps not difficult. Carlson (14) has demonstrated that the digestive tract of cats and dogs in parathyroid tetany is depressed or paralyzed. This is particularly true of the motor mechanism but there is also a secretory depression. If then parathyroid tetany produces also a paralytic ileus with stasis and increased absorption of toxic products from the intestine, and along with this there is pronounced anorexia and vomiting, the difficulty of controlling the situation by dietary measures alone becomes obvious. Such animals can however be restored to good condition and will then take the special diet if the toxemia is relieved by Luckhardt's method, namely, the intravenous injection of large amounts of Ringer's solution.

CONCLUSIONS

1. Parathyroid tetany or depression is due to an intoxication.

2. The responsible toxic substances come chiefly from the gastrointestinal tract. They arise through the activity of the proteolytic group of intestinal bacteria, and are probably for the most part protein split products of the nature of amines.

3. The function of the parathyroid glands is to prevent intoxication by these poisons.

4. The parathyroid glands do not furnish a hormone necessary for life, and dogs may be kept alive indefinitely after their removal if treatment directed to the prevention of this toxemia of intestinal origin is carried out.

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THE CONDITIONS OF SURVIVAL OF MAMMALIAN NERVE TRUNKS

A. FORBES AND L. H. RAY

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In the course of certain researches in this laboratory, some of which have been completed and published (1), (2), (3) and some of which are still in progress, it has been found useful to excise mammalian nerve trunks and record their action currents produced in response to artificial stimulation. Practically all the classical investigations of the nerve impulse have been made on the sciatic nerve of the frog, and yet for certain purposes a mammalian nerve is a far better preparation. The sciatic nerve of the frog is so small that its ohmic resistance per centimeter is very high; and in the average frog the total length of the nerve is not more than 7 cm. In the cat the sciatic nerve, which is large and easily handled, can readily be dissected out from the hip to the popliteal space, and in this region a length of 8 or 9 cm. is found free from branches. With a sharp scalpel it is easy, without damaging it appreciably, to split the sciatic nerve in this region and dissect the peroneal branch away from the rest of it to a point above the hip joint, thus obtaining a nerve trunk free from branches and 13 or 14 cm. long. If a greater length is desired, the tibial nerve can be dissected as far as the ankle. By cutting the sciatic nerve behind the hip and making this dissection a total length of nerve amounting to 22 cm. or more can be had. In the dog a correspondingly longer nerve can be had with the same dissection.

For a number of purposes these preparations are far superior to the frog's nerve. In the study of action currents it is possible to place the stimulating electrodes far enough away from the leading-off electrodes to minimize the electrical artefact (2, p. 189) resulting from powerful stimuli, often a serious source of confusion in the frog's nerve. When the velocity of the nerve impulse is to be studied a great length of nerve is obviously useful. The comparatively low electrical resistance of these larger nerves enables one to obtain larger excursions of the

galvanometer in recording the action currents. Furthermore in certain researches on mammalian reflexes it is important to control the results by comparing reflex responses with those obtained from the afferent nerve used for producing them; and this can only be done by recording the action currents in the afferent nerve. To do this it is usually best to remove the nerve trunk from the body, and in evaluating the results of this procedure it is of some importance to know the conditions of survival of the mammalian nerve trunk after the removal, and whether the tissue can be considered functionally normal. Gotch (4) states that the demarcation current in a mammalian nerve declines slowly if the exposed nerve remains "in structural and physiological connection" with the rest of the tissue, but more rapidly on the death of the animal or complete excision of the nerve.

In many experiments in the laboratory a branch of the sciatic nerve (e.g., peroneal) has been removed from a decerebrate cat and either set up at once in a moist chamber or kept in mammalian Ringer solution[1] at about 20°C., and has been found after 2 or 3 hours to manifest action currents not differing appreciably from those which were obtained immediately after removal from the animal's body (1), (2). In several instances action currents have been obtained from these nerves after they had been kept for days in Ringer solution; in four cases they were recorded in nerve 4 days after removal from the animal (see fig. 5). Usually the nerves kept over-night have been maintained at a temperature of about 5 to 10°C., but in one instance the nerve was kept between 15 and 20°C., and still showed action currents. In general when the nerve was kept over-night the action currents were appreciably reduced in magnitude, but otherwise did not appear to differ in character from those obtained from the fresh nerve.

The fact that the function of a mammalian nerve trunk, as judged by the action current, persists unimpaired for many hours after its removal from the body, seems to indicate that the nerve is to an extraordinary degree independent of its blood supply, as compared with mammalian muscles, which cease to function very soon after the blood supply has been cut off. One might, therefore, suppose that nerves remaining within the animal's body after death would retain their functional capacity to the same extent as those that have been removed from the body. It was surprising to find that the sciatic nerve carefully

[1] The Ringer solution was made up according to the following formula: NaCl, 0.9 per cent; KCl, 0.042 per cent; $CaCl_2$, 0.024 per cent; $NaHCO_3$, 0.02 per cent.

dissected from a cat or dog an hour or two after death showed no trace of action current when connected with the galvanometer and stimulated in the usual way. After this observation had been repeated a few times we inferred from the apparent contrast it afforded with the prolonged survival of nerves outside the body, that the environment within the body after death, must be less favorable to the survival of the nerve than Ringer solution or air in a moist chamber at the same temperature, an inference we have had to modify, as will presently appear. In a few instances, nerves removed from anesthetized or decerebrate animals in a living condition have failed to show action currents when connected with the galvanometer a few moments later. Both on account of the practical need of knowing the conditions under which mammalian nerves could be relied on to retain their normal function after removal from the body, and on account of the theoretical significance of these conditions, it seemed worth while to carry out a large series of experiments to clarify the problem.

In a laboratory where mammalian material is extensively used and where a string galvanometer is permanently installed and wired for recording action currents, it is easy to collect a considerable number of observations with great economy of time and material, by using animals after they have served for other experiments. More than two hundred nerves removed from animals, either living or recently killed, have been subjected to various conditions and examined with respect to survival of function. We do not feel that we have established with certainty all the conditions of survival; there have been certain puzzling instances of failure to function which we could not explain; but there are several points which we believe we have definitely established, and it seems to us worth while to present a statement of these, even though the problem as a whole is not as completely settled as we could wish.

METHOD. Almost all the nerves examined were from cats; a few were from rabbits and dogs. Almost all were taken from animals which had previously been used for other purposes, comprising a great variety of research experiments and class demonstrations and exercises. In every case the animal had been anesthetized at the outset. In some cases the animal had been decerebrated under ether and kept in a decerebrate condition until the ether was eliminated from the system; in others the animal had been anesthetized with urethane, and consequently had remained in a state of anesthesia throughout the experiment. Some nerves were removed from the animals while they were still living, both by way of control, and in order to examine the effect of

varying other conditions than those arising in the dead body. In other cases the nerves were removed from the body of the animal at various intervals after death, up to 19 hours. Death was caused by a variety of procedures, including extensive bleeding, and asphyxia by opening the thorax. In some experiments the nerve was set up in a moist chamber; in others it was merely connected with stimulating and leading-off electrodes in the open air, and observations were made rapidly in order to prevent the nerve from drying. In most cases the nerve was crushed at a point between the leads to render the action current monophasic. The distance between the leads was about 2 cm.

Stimulation was with platinum or silver electrodes connected with a Berne inductorium calibrated for break shocks in accordance with Martin's scale (5). For leads we used the non-polarizable porous "boot" electrode kept soaked in Ringer solution, and filled during the experiment with zinc sulphate in which were dipped amalgamated zinc rods. In a few of the later experiments these electrodes were replaced with others of silver coated with AgCl. For recording the action currents we have regularly used a string galvanometer. In the first part of the series the galvanometer used was one made by the Cambridge Scientific Instrument Co.; during the latter part of the series we used a Hindle galvanometer with a 1.5 mm. air gap.

In adjusting the tension of the string it must be recognized that no string galvanometer has been made which can record electrical changes as rapid as those in the action current of a medullated nerve (6), without a lag which grossly distorts the resulting curve (1, fig. 7). The peak of the electrical change is over before the string attains its maximum deflection, even when the string tension is increased to the point of causing marked periodic oscillations. For the purpose of making records with the least possible distortion it is desirable to use a tension which brings the string to the limit of periodicity, as shown by a very slight "overshoot" in the calibration curve produced by the make of a constant current. Einthoven's method of condenser damping (7) can be used to increase the tension at which this condition is reached, and thus to reduce the distortion of the record. But with a fine string such as is best for nerve work because of its small inertia, at a tension sufficient to give the greatest accuracy in photographic recording, the deflection is so small and the return to the resting position so rapid, that it is difficult to tell by watching the shadow whether it reveals an action current or not. If, on the other hand, the string is slack it is usually easy to determine by eye the presence of action currents and to dis-

tinguish them from electrical artefacts (2, p. 189), unless the action current is very small and the threshold of stimulation so high that excitation only occurs with stimuli producing artefacts of considerable size. A slack string responds to an action current in the manner of a ballistic galvanometer as compared with a tight string. If the action current be monophasic the deflection will be relatively large and prolonged (1, fig. 7), and the eye can readily discern its direction, which is essential in distinguishing action currents from artefacts.

In adjusting the tension the most convenient measure is the deflection of the shadow in millimeters caused by a millivolt applied directly to the string; i.e., with no resistance in series. As long as the same string is used and the magnification is kept constant, this measure serves very well for designating the tension; but if the string is replaced by one of different resistance, or if the magnification is altered, a calculation becomes necessary to compare any given tension under the new conditions with a designated tension under the old conditions. It is useful, therefore, to find a simple standard of tension which shall be constant and absolute for a given galvanometer. This is easily done, provided the magnetic field be kept constant, by expressing the tension in micra of absolute deflection of the string (not the shadow) per micro-ampere of current passed through it. This is the same as meters per ampere, although, of course, the greatest actual deflections of the string and the greatest current traversing it are but minute fractions of these latter units. The tension in these absolute units is readily calculated from the observed deflection by the following formula: $T = \frac{DS}{M}$, where T is the tension expressed in meters per ampere, D, the deflection of the magnified shadow in millimeters per millivolt, S, the resistance of the string in ohms, and M, the magnification. This way of expressing tension affords a permanent standard for a given galvanometer as long as the field is constant. As long as the same string is used and the magnification is unchanged, the expression $\frac{S}{M}$ will remain a constant by which the observed deflection in millimeters per millivolt is multiplied in order to obtain the absolute tension. The numbers in which the tension is expressed are of a convenient order of magnitude; e.g., the range of tensions used for recording the action currents of nerves we found to be from about 30, which is very tight, to about 500, which is slack. We have found that in the Hindle galvanometer without condenser damping a gilded quartz string of 1.5 micra diameter had its limit of

periodicity at about 65 meters per ampere, whereas a similar string of 2.75 micra diameter is periodic unless it is slacked to a tension of about 150.

In examining nerves for survival of function, stimuli of various strengths were used, and responses to both make and break shocks were observed. This is important, for the surest test of an action current lies in the fact that the deflection is in the same direction on both make and break shocks, whereas the electrical artefact is always in opposite directions on make and break shocks (see fig. 3, B). Certain electrical artefacts due to capacity effects are not reversed by reversing the wires from the secondary coil, but all are reversed between make and break.

Photographic records were obtained in a large majority of experiments. In a few with a slack string reliance was placed on recognition of action currents by eye.

Various special procedures will be described in connection with the observations.

RESULTS. Disappearance of function in those nerves which remained undisturbed in the body of the animal after death was generally found to be complete in 3 or 4 hours. This is illustrated in table 1, based on a group of the earlier experiments. In appraising these results we should note that of 80 nerves removed before death, 69 (86 per cent) showed action currents. The problem of explaining the remaining 14 per cent which failed to show function will be discussed presently; for the present it must suffice to mention the observed percentage of nerves with surviving function as a control for the rest of the series. Of all the nerves removed from the body in good condition before death or within an hour after, and placed in Ringer solution at room temperature, more than 60 per cent retained their functional capacity for 4 hours or more. Among those surviving as long as this, some were from animals under urethane, some under ether, and some from decerebrate animals from whose systems the anesthetic had been eliminated. It seemed to make little difference to survival outside the body, which of the three conditions had obtained prior to excision.

In apparent contrast with this prolonged survival in Ringer solution the evidence presented in table 1 illustrates the comparatively rapid disappearance of functional capacity within the dead body.

In the first column are shown the times of excision of the nerves after the death of the animal, in 1-hour groups. The time of death is taken as the moment when effective heart beats ceased (fibrillation was sometimes seen for some minutes afterwards). In the second and

third columns are the numbers respectively of those that did and those that did not show action currents when first tested after excision. In this table we have included only those experiments in which the nerve was tested within 30 minutes after excision; almost all were tested within 15 minutes. In some experiments the nerve was not tested for more than half an hour; these have been omitted from the table for reasons which will appear later. In the final column are the percentages showing action currents in each group.

Although the observations thus far presented show definitely that mammalian nerves removed within an hour after death generally survive in Ringer solution more than 4 hours after removal from the body, and that those nerves left undisturbed in the body cease to show function in less than 4 hours, still the apparent contrast proved on further investigation to be somewhat misleading. We found that temperature exerts a profound effect on the survival of nerve. The air temperature

TABLE 1

TIME OF EXCISION	NUMBER OF NERVES SHOWING RESPONSE	NUMBER OF NERVES SHOWING NO RESPONSE	TOTAL	RESPONSES
hours				*per cent*
0–1	19	5	24	79
1–2	7	4	11	63
2–3	8	4	12	67
3–4	1	6	7	14
4–5	0	3	3	0

in the laboratory was usually about 20°C. The nerve, placed in a beaker of Ringer solution beside the animal, immediately acquires the temperature of the surrounding air. The cooling of the body of the animal in the same room is so slow that for many hours the nerve within the body is at a considerably higher temperature than the control nerve. A series of curves, reproduced in figure 1, each showing the decline of temperature within the body of an individual cat after death, illustrates this point. We also found that our preliminary observations had not been carried far enough, in that failure of the nerve to manifest function on removal from the body had been taken to indicate death of the tissue, and the nerves had been promptly discarded and their subsequent history not observed. To make an adequate comparison, therefore, we found it necessary to adopt the following procedure: Shortly before or immediately after the death of the animal the sciatic nerve was dissected out from one leg and put in a tube so constructed that it could be

sealed with wax. This tube was then filled about half or two-thirds full of Ringer solution, placed in the wound between the muscles in the neighborhood from which the nerve had been removed, and the wound sewed over it. The animal was then left for the desired time. In this way the control nerve and the nerve left *in situ* in the other leg were enabled to cool together at practically the same temperature. At the end of the time to be tested the sciatic nerve from the other leg was dissected out and placed on electrodes in a moist chamber, to be examined for action currents. At the same time, or immediately after a record was taken, the control nerve (in the tube) was removed from the wound, taken out of the tube, and placed on the electrodes. For reasons which will presently appear (see page 453), care was taken to place the stimulating electrodes as nearly as possible at the same distance from the leads in the case of each nerve. The leads were kept

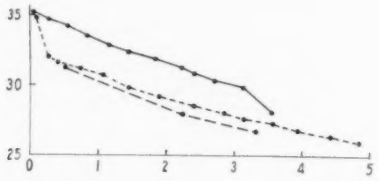

Fig. 1. Decline of temperature in three cats after death. Air temperature about 20°C. in each case. Temperature by rectum in the case shown by the solid line; in the other two cases the thermometer was between muscles of leg. Abscissae, time in hours; ordinates, degrees C.

the same distance apart in order to maintain equal conditions in the nerve-galvanometer circuit. To avoid error due to spread of the effect of injury (2, p. 179), the stimulating electrodes were always applied at least 12 mm. from the cut end of the nerve. When galvanometer records had been made, both nerves were placed in a beaker of Ringer solution at room temperature, and from time to time tested again. During the night (and sometimes also during most of the day-time) they were left in a cold place where the temperature was between 0° and 10°C. Readings were made at various intervals during the following day, and on succeeding days, until the nerves showed no response to stimuli.

In order to interpret the results of this procedure, we must digress to the extent of noting the effects of temperature on the responses observed in mammalian nerves. Britton (8) reports that in mammals peripheral nerves show function from 1 to 2 hours after death induced by cold, the

temperature at death being about 16°C. As we shall presently see, the temperature at which the nerve is kept is a most important factor in determining the time it will survive. But also the temperature of the nerve at the moment when it is tested influences the magnitude of the response. In tracing the persistence of functional capacity, it is therefore necessary to control the temperature at the time when the observations are made. Nor is this all; the degree and duration of cooling appear to affect the speed of recovery. In a prolonged experiment, of which a graphic record is shown in figure 2, the sciatic nerve of a cat, removed 25 minutes after death, was placed in a moist chamber in which the temperature could be regulated, and action currents were recorded as the temperature was alternately raised and lowered. The moist chamber was similar to that designed by Keith Lucas (9). It has been

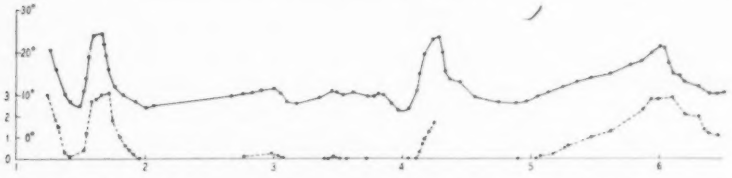

Fig. 2. Effect of temperature on size of action current in mammalian nerve. Abscissae, time in hours after removal of nerve from body. Ordinates: for solid line, temperature in degrees C.; for broken line, galvanometric excursion in millimeters of monophasic action current on maximal stimulus. Hindle galvanometer; resistance of string, 17,000 ohms; magnification 490; tension adjusted to 174 m. per amp. at beginning, gradually tightened to 94 at end. Individual observations shown by round dots.

described in part in a previous communication (10); a feature not previously described is a water-jacket enclosed between an inner and an outer glass cylinder, whereby the temperature within the chamber is uniformly altered by the current of cold or warm water flowing through the jacket. This water-jacket being transparent, it is easy to note the temperature of the air within by means of a thermometer placed close beside the nerve.

Starting at room temperature, the nerve was three times cooled till the action currents disappeared, and each time was subsequently warmed; finally the temperature was lowered slowly to 10°C. and the experiment was discontinued. Two points of interest are shown by this experiment (see fig. 2): first, at temperatures below about 8°C. the action current disappears; above about 15°C. the excursions are all of approximately the same size; between these two values the size of the

excursion appears to be a function of temperature. The disappearance of the electric response at temperatures below 8° presents a notable contrast with amphibian nerves which show responses at much lower temperatures than this. The second point is the lag in the change of electric response behind the change of temperature which induces it. This lag may be due in part to the time required for the change of temperature to become established throughout the nerve, but it can hardly be wholly due to this, for it evidently depends to a considerable extent on the time the nerve has been kept cool, or else on the temperature to which it has been cooled or on both. The first time this nerve was cooled it remained below 8° for about 4 minutes; when it was warmed after this a substantial increase in the action currents had already appeared when the temperature reached 15°. The second time the nerve was cooled it was kept cold for 2 hours and was below 8° for 10 minutes before it was warmed, and the minimum temperature was slightly lower than before; this time when the temperature reached 15° the return of the action currents was much less marked than after the first cooling. This difference might conceivably be due to the more thorough chilling of the nerve in the second case, but the presence of a delayed effect is further substantiated by other experiments in which a nerve kept cool over night required a considerable time after being warmed to room temperature before showing full-sized action currents. In one instance a tibial nerve, kept 18 hours in cold Ringer solution, (8.1°C. at the end of this time) was then transferred to another beaker of Ringer solution at 18.2°. In 13 minutes it was tested and showed only a very slight action current; 25 minutes after warming it was tested again and showed a greatly increased action current. In another experiment a sciatic nerve was kept 20 hours in cold Ringer solution (6.8° at the end of the time); it was then placed in a moist chamber at 16.8° and tested from time to time; in 7 minutes there was no response, at 27 minutes there was a doubtful trace of electric response, at 46 minutes there was a small response (0.3 mm.), at 55 minutes the excursion had reached a maximum (0.9 mm.) beyond which it did not go. In still another experiment a pair of nerves which had been kept for hours in Ringer solution at 1.0°C. were raised gradually during an hour to 4.7°, then transferred to another beaker of Ringer solution between 15° and 16°; 50 minutes after the transfer the maximal action currents gave galvanometric excursions of 0.9 mm. and 2.1 mm., respectively; 2 hours after the transfer the excursions in response to the same stimuli were 1.6 mm. and 4.4 mm., respectively.

These observations indicate that prolonged cooling modifies the nerve in such a way that it requires a longer time for function to reappear on warming than it does after cooling of brief duration. We do not claim to have worked out this point with any quantitative accuracy, nor to have determined how much the delayed recovery depends on the duration and how much on the degree of cooling; it has been dealt with as an incidental issue on account of the precaution which it indicates, to allow plenty of time after warming before making tests for quantitative comparison.

Returning to the comparison between nerves lying undisturbed in the body after death and nerves kept in Ringer solution at the same temperature, we may now examine the results of our experiments, bearing in mind the necessity of avoiding confusion due to changes of temperature. Ten complete experiments on this point were carried out by the method described above. In the first five of these, the control nerve was placed in a tube whose capacity was 8.5 cc. We found that when the nerve was thus enclosed for more than 3 hours (the time needed to insure the disappearance of function in the nerve left *in situ*) the action currents recorded immediately on removal from the tube were either absent or very small compared with the usual responses of nerves kept in Ringer solution. This fact and the subsequent recovery of function in a beaker of Ringer solution, led us to conclude that the tube was too small to contain enough oxygen to simulate the conditions in a beaker exposed to the air. We therefore replaced this tube in the next five experiments with one of 36 cc. capacity. The result of the change justified our conclusion; in every case when the large tube was used the nerve showed a substantial action current immediately after removal.

All of the ten experiments showed that the nerves left undisturbed in the tissues (in two cases as long as $4\frac{3}{4}$ hours after death) recovered their functional capacity when placed for a time in Ringer solution. Responses illustrating this point are reproduced in figure 3. They showed, furthermore, that in almost every case the nerves which had been left *in situ* survived better than the controls. In seven of the ten experiments (including four of the five with the larger tube) the control nerve (that in the tube) showed the larger responses during the first few hours after removal, but when tested more than 20 to 27 hours after death the nerve left *in situ* showed the larger reponse of the two. In one of the three remaining experiments the same point was illustrated by the fact that the curves of response converged, although they

didn't actually cross; that is, the responses of the "tissue" nerve increased after the two nerves had been placed in Ringer solution together, while those of the control decreased; but in this case the "tissue" nerve responses never became larger than those of the control. In the other two experiments the curves of response in the two nerves ran approximately parallel courses. Curves from three experiments, showing the variations in the size of response plotted against time, are reproduced in figure 4. It should be understood that to avoid confusion the curves are based only on those observations which were made after the nerves had been at room temperature long enough to complete the recovery from chilling, described above, and that these were made at the infrequent intervals shown by the dots; therefore, the lines connecting the dots in the figure may not express accurately the values which the responses would have had if recorded more frequently. The three experiments from which these curves were made were those in which the nerves were left longest *in situ* (between $4\frac{1}{2}$ and 5 hours), and, therefore, serve best to bring out the difference between the two conditions; in all of these the large tube was used for the control.

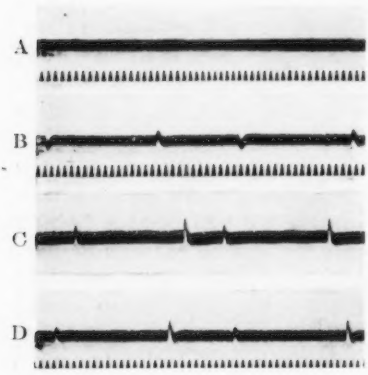

Fig. 3. Galvanometer records of action currents, showing recovery in Ringer solution of nerve which has lain undisturbed in the body for 3 hours and 48 minutes after death. A, B and C, left sciatic nerve; A and B, 3 minutes after removal; C, after nerve had been in Ringer solution for 17 hours between 8° and 10°C., for 3 hours at 19°C.

Stimuli: alternate make and break shocks; coil distance such that break shock in A is 310 Z units; in B, 750 Z (showing artefact); in C, 230 Z.

D, control nerve (right sciatic) removed 15 minutes after death; in Ringer solution $3\frac{3}{4}$ hours. Break shocks, 57 Z.

Speed of film the same in all, shown in A, B and D by tuning fork record of 100 vibrations per second.

Hindle galvanometer, 17,000-ohm string; magnification 490, tension 94 m. per amp.

The fact revealed by these experiments is that although remaining undisturbed in the body after death causes in a nerve trunk a suspension of function which does not occur in a nerve kept in Ringer solution at the same temperature, the life of the nerve is thereby prolonged. In the suppression of function there seems to be some measure of protec-

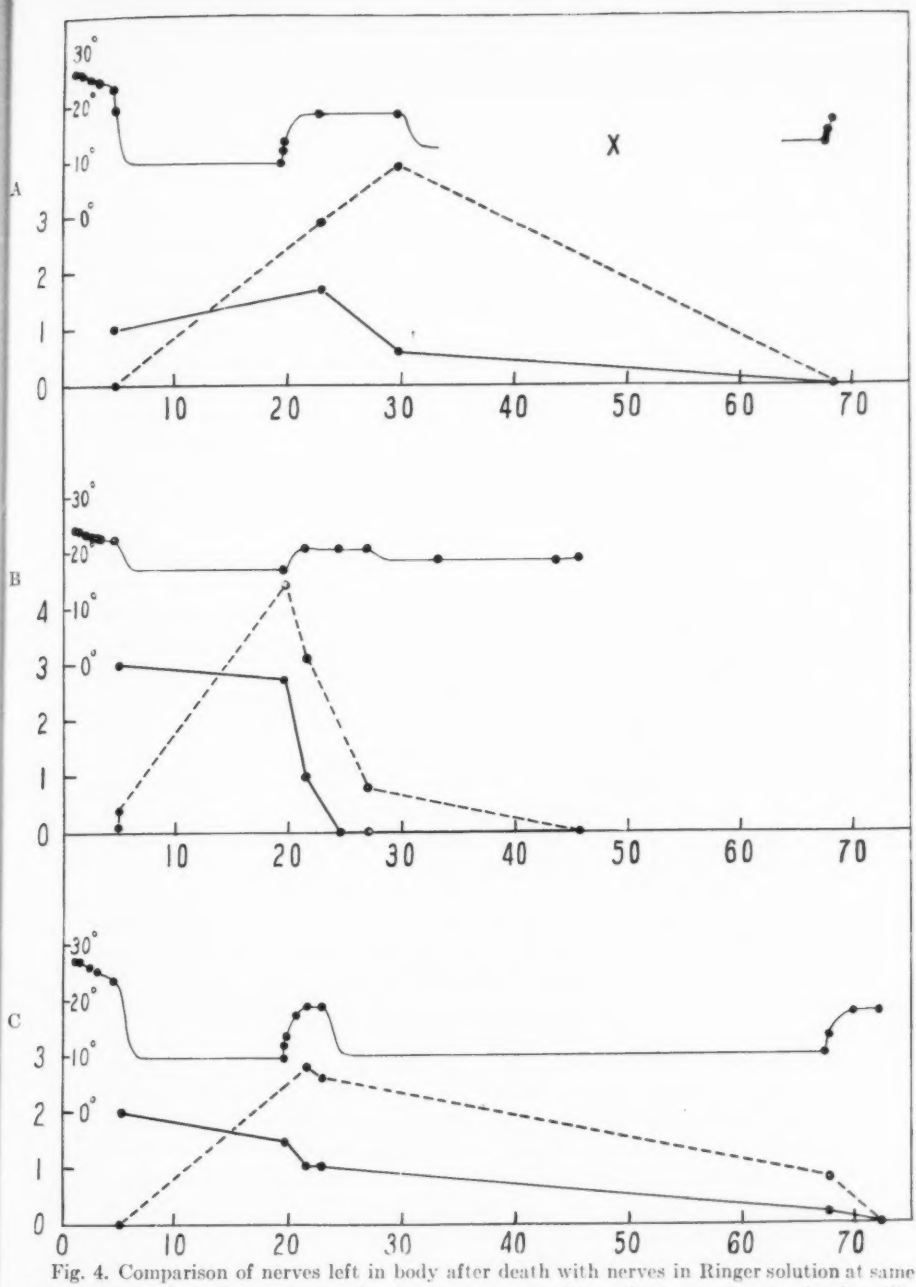

Fig. 4. Comparison of nerves left in body after death with nerves in Ringer solution at same temperature. In each experiment the two lower curves show galvanometric excursion in millimeters of monophasic action current with maximal stimulus: broken line, "tissue" nerve: solid line, control nerve (see text). Records taken with Hindle galvanometer, 20,000-ohm string; magnification 490; tension in A, 164 m. per amp.; in B and C, 205 m. per amp.

Uppermost curve shows temperature in degrees C. Abscissae, time in hours after death. Individual observations shown by round dots.

A, "tissue" nerve left 4 hours and 39 minutes after death; control nerve removed 5 minutes after death. During 36-hour interval marked *X*, temperature outdoors rose to 25°C.; nerve in beaker of Ringer solution close to window; no observations made in this time. B, "tissue" nerve left 4 hours and 49 minutes after death; control nerve removed 32 minutes after death. C, "tissue" nerve left 4 hours and 45 minutes after death; control nerve removed 45 minutes after death.

tion from the permanent cessation of function which constitutes death. This protection, however, is not without its limitation, for in two experiments nerves left undisturbed in the body beween 19 and 20 hours, and then carefully dissected out, showed no trace of response, although kept under observation more than 4 hours after removal—in one case 24 hours—and tested repeatedly during that time. Since in several experiments nerves have survived 4 days when kept in Ringer solution below 10°C. (fig. 5) we infer that though the environment of the dead body during the first 5 hours after death promotes prolonged survival of the nerve better than Ringer solution at the same temperature exposed to the air, it does not protect the nerve from death as well as Ringer solution below 10°C.

Effect of temperature. It has already been stated that temperature exerts a profound influence on nerve survival. We may now summarize the evidence on this point. Many times we have left nerves over-night in Ringer solution at room temperature, and next morning, usually from 16 to 22 hours after excision from the body, have tested them and found in them no trace of response. In only three cases did nerves kept at room temperature over-night show action currents the following morning. These three nerves showed responses up to 17, 22 and 24 hours, respectively, after death. That which survived the longest had been left in the body $3\frac{3}{4}$ hours after death, then on dissection was placed in a moist chamber and left there through the night. The other two were kept in Ringer solution. In all three the temperature was about 18°C. When nerves freshly dissected and in good condition were placed in Ringer solution between 0° and 10°C. they almost uniformly showed good responses on being warmed the following morning; that is, from 18 to 22 hours after the death of the animal. Eight nerves thus treated showed responses after 48 hours; four of these after 88 hours. In most of these tests of prolonged survival the nerves were kept cold about 80 per cent of the time. We have not tested the effect of lowering the temperature of nerves below freezing. The only case of mammalian tissues surviving outside the body for a time greatly in excess of this, that we know of, is that of a human pulmonary artery which after being kept in Locke's solution in an ice chest 49 days responded to epinephrin (11).

In four experiments a comparison was made between the survival of nerves at room temperature and at body temperature. In each of these experiments two corresponding nerves (right and left peroneal or right and left popliteal) were dissected out of the animal before their

function had disappeared, in every case in less than 3 hours after death. In each case the nerves, as soon as dissected, were placed in separate beakers of Ringer solution, one at 37°C., one at 19°C. They were alternately taken out and placed in the moist chamber for test from time to time for about 4 hours after dissection. The tests were made rapidly and the nerves returned at once to Ringer solution, so that the interruption of the designated temperature was usually not for more than 5 minutes.

In every case the responses ceased in the warm nerve considerably sooner than in the cool one. In the warm nerves the disappearance of function varied from 50 minutes to $2\frac{1}{2}$ or 3 hours after dissection; in the cool nerves it varied from $2\frac{1}{2}$ hours to more than 4 hours. The course of a typical experiment is shown in figure 6. In this experiment both

Fig. 5

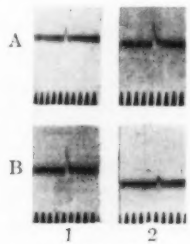

Fig. 6

Fig. 5. Action currents of sciatic nerve of cat, recorded 4 days after removal. Nerve removed $3\frac{1}{2}$ hours after death of animal, and left in Ringer solution at a low temperature (5.3 to 8.5°C.) for the greater part of the time; put in Ringer solution at room temperature 25 minutes before this record was taken. Break shocks, 76 Z. Hindle galvanometer, 17,000-ohm string; magnification 490; tension 139 m. per amp.

Fig. 6. Action currents showing effect of temperature on nerves of cat. Popliteal nerves removed 1 hour after death of animal. A, in Ringer solution at 19°C., B, at 37°C. *1*, after 10 minutes in Ringer; stimulation, 122 Z; *2*, after $1\frac{3}{4}$ hours in Ringer; stimulation, 174 Z.

Hindle galvanometer; 25,000-ohm string; magnification 490; tension 102 m. per amp.

nerves were left undisturbed in the body for an hour after death. The first two records show the action currents of the two nerves in response to maximal stimulation soon after removal from the body; the nerve which has been in Ringer solution at body temperature shows a larger action current than that which has been at room temperature. The last two records show the responses of the same nerves about $1\frac{3}{4}$ hours

later; the responses of the nerve which had been kept at 37°C. have now become very small, while those of the nerve at room temperature have increased in size.

It is evident that the cooler a mammalian nerve is kept (within the temperature limits we have tested) the longer it survives. At temperatures between 0° and 10° it lasts much longer than between 15° and 20°; at 37° (body temperature) survival is much shorter than at 20°. There is an interesting resemblance between the effect of lowering the temperature below 8° and that of leaving the nerve *in situ* in the dead body;—in each case function is suspended while the condition is maintained, but (within limits) survival is thereby prolonged.

Contact with injured tissues. In certain experiments on mammalian reflexes it has been necessary to expose peripheral nerve trunks for considerable distances, and then to repeat observations at somewhat prolonged intervals of time. Sometimes it has been deemed expedient in such experiments to protect the nerve by closing the wound over it between observations. In one of these experiments on a decerebrate cat it was found that after the nerve had lain for 3 hours between the muscles which had been dissected apart to expose it, no response could be obtained from it on stimulation. Contact with the muscles which normally surround the nerve appeared, instead of protecting it, to have abolished the nerve's function.

In several experiments the effect of the same procedure was tested, the experiments being controlled by comparing the nerves so treated with others kept for the same time in Ringer solution at the same temperature. Twelve experiments of this sort were performed on the sciatic nerves or their branches in decerebrate cats. In most cases the nerves compared were the corresponding nerves from the two sides; in three experiments the peroneal and popliteal nerves of the same side were compared, these being nearly enough of the same size to serve fairly well for this sort of comparison. In some cases equality of temperature between the two nerves was maintained by keeping a thermometer with its bulb enclosed in the wound beside the nerve, and keeping the beaker containing the control nerve in Ringer solution at the same temperature by placing it over a flame when necessary. In the later experiments we used the same procedure that was adopted in the case of the dead animal; viz., the control nerve was placed in the tube described above, half full of Ringer solution raised to the proper temperature at the start, sealed with wax and enclosed in a wound in the leg. The time that the nerve lay between the dissected muscles was varied

in different experiments from 35 minutes to 2 hours. The temperature of the nerve between the muscles varied in different experiments from 28°C. to 38°C., sometimes it varied as much as 7° or 8° during a single experiment, but in every case the average temperature of the control nerve did not differ by more than about 1° from that of the nerve lying in the tissues, and in general we made a point of keeping the temperature of the control above rather than below that of the "tissue" nerve.

It has already been noted above that when mammalian nerves are removed from their blood supply and kept in Ringer solution at body temperature (37°C.) their function disappears in from 50 minutes to 3 hours. Therefore, it was to be expected that in this series in which the tests were carried on sometimes for 2 hours, a certain percentage of the controls would have ceased to function at the end of the time. This was the case in one experiment in which the two nerves were kept for an hour between 38° and 39°C.; at the end of this time neither the nerve in the tissues nor the control showed any response to stimulation. In another experiment small galvanometric excursions were obtained from both nerves, but we could not be certain that they were not due to artefact ("escape of current"). In a third experiment in which the nerves were enclosed in the wounds for 1 hour and 50 minutes, the limbs varying in temperature from 30° to 26°C., neither nerve showed impairment of function, both threshold and size of response being normal in both nerves at the end of this time. Of the remaining experiments all but one showed a distinct loss of function in the nerve lying between muscles as compared with the control, the responses being absent in three cases, doubtful in two cases, and much smaller than the responses of the control in the other three cases. The only experiment showing the opposite result was one in which the "tissue" nerve (popliteal) was enclosed in the wound for 1 hour and 40 minutes at a maximum temperature of 38°C., while the control (peroneal) was kept in warm Ringer solution for 1 hour and 46 minutes at a maximum temperature of 39°C. When tested the responses of both nerves were almost completely abolished, and those of the "tissue" nerve were only slightly larger than those of the control. Since the nerves were not mates, and since the temperature and the time it was maintained were greater in the case of the control nerve, and sufficient to account for its loss of function, this exceptional result has little weight in opposition to the majority. These results, then, confirm the observation that a nerve cut off from its blood supply and lying in a wound, completely surrounded by dissected tissues, instead of being thereby kept in normal condition,

loses its capacity for response more rapidly than if lying in Ringer solution at the same temperature. In five experiments the same procedure was tried in cats under urethane, turned over to us still alive at the conclusion of various experiments which other workers in the laboratory had performed. These experiments showed that the loss of function was usually not so rapid in the animals under urethane as in the decerebrate animals, from whose systems the general anesthetic had been eliminated. In these experiments the nerve lay in the tissues $1\frac{1}{2}$ to 2 hours after dissection, but in only one case was its function wholly abolished; in another case the response was reduced to a much smaller value than that of the control, but in the remainder there was very little difference between the two.

Recalling the fact that in the case of the nerve lying undisturbed in the body after death disappearance of function proved (within limits) to be temporary, and indeed associated with an actual promotion of subsequent survival, the question now arises whether the cessation of function which follows surrounding the nerve with dissected tissues during life, was also temporary and whether it might also favor survival. In some of the experiments just described these points were not tested; in the later experiments they were. In six of the experiments on decerebrate animals and in the two urethane experiments showing impairment, the question of recovery was tested. In all of these the nerves which had lain in contact with the tissues showed recovery of function, either by reappearance of a response which had disappeared altogether, or by increase in the size of a response which had become very small, or (in one case) by the lowering of threshold. In two instances the recovery was observed while the nerves were set up in the moist chamber, not having been in contact with Ringer solution at any time. In the rest of the experiments the nerves, after their first test, were placed in Ringer solution and recovery was observed when they were later returned to the moist chamber.

In four of the six decerebrate experiments showing recovery, an attempt was made to test the second question; that is, whether prolonged survival was promoted by leaving the nerves between the muscles as it was by leaving the nerve *in situ* after death. As usual, for these tests of survival the nerves were kept through the night in Ringer solution at about 10°C. The results were not wholly concordant. In one carefully controlled experiment with a decerebrate preparation in which the responses of the "tissue" nerve had almost disappeared, but had recovered well in the moist chamber, the responses of this nerve on the

following day were considerably larger than those of the control. This experiment, therefore, appeared to indicate a similarity of the condition of the nerve so treated with that of the nerve left *in situ* for 3 or 4 hours after death, the temporary suppression of function being followed by increased survival. In the other three experiments the tissue nerves continued to show smaller responses than the controls throughout the course of the experiment. We concluded that the variable factors of blood supply and degree of injury to the surrounding tissues, which are difficult or impossible to control, probably influenced the result and rendered it inadvisable to pursue further this particular phase of the problem.

Apparently in two respects the effects of lying completely covered by dissected muscles in the living animal and of lying undisturbed *in situ* after death are similar; namely, in each case cessation of function occurs, and in each case, if the condition is not maintained too long, recovery may occur. The lack of uniformity in the effect of an environment of injured living tissues on subsequent survival suggests that in this case, as in the case of death of the animal, some factor is present which tends to promote prolonged survival of the nerve, but that probably other factors enter in varying degree, which in certain cases more than offset the effects of the first factor. The possible nature of these factors will be discussed presently.

Conduction with a decrement in surviving nerves. In some of the earlier experiments in which a nerve was left undisturbed in the body for 2 or 3 hours after death, we found that on removal an electric response could be obtained if the stimulus was applied within 3 or 4 cm. of the leading-off electrodes, but if applied farther away, no response occurred. This might result either from a definite and absolute block in conduction at the point beyond which stimulation failed to produce a response, or it might result from conduction of the propagated disturbance with a decrement throughout the entire length of the nerve, the impulse being able to travel without extinction, for instance, 4 cm. but not 5 (cf. 12). To determine which of these two possible explanations was the true one the following experiment was devised. We applied to the nerve to be tested four pairs of electrodes, two pairs of stimulating electrodes and two pairs of leads, as shown in figure 7. The nerve could be stimulated at either the proximal or the distal pair of stimulating electrodes and the response could be tested at either the proximal or the distal pair of leads. If stimulation with the proximal electrodes evoked a response at the proximal leads, and stimulation with

the distal electrodes evoked a response at both proximal and distal leads there could be no absolute block at any point in the nerve. If, then, stimulation with the proximal electrodes failed to evoke a response at the distal leads it would prove that the nerve impulse was conducted with a decrement. For this experiment we used the popliteal branch of the sciatic dissected centrally as far as the hip and its peripheral extension as the tibial nerve dissected distally as far as the ankle. In this way we obtained the maximum available length of nerve,—in some cases more than 20 cm. Nineteen of these experiments were performed on nerves which had lain undisturbed in the body after death, for a time which was varied in different experiments. In some experiments conduction occurred without appreciable decrement; in some the total failure of conduction in part of the nerve, due to accidental damage, prevented the demonstration of the effect. In seven cases, in which the time the nerve lay in the carcass was between 1 and 3 hours, conduction with a decrement was clearly demonstrated; the results of a typical experiment are shown in figure 8. The reversal of direction of the galvanometric deflection when the stimulus was applied at the distal electrodes and the response recorded at the proximal leads is due to the reversal of the direction of the nerve impulse and consequently of the order in which the two electrodes are reached by the disturbance. For comparison there are shown in figure 9 records similarly obtained (except that the nerve was crushed between the leads to render the action currents monophasic) from a nerve in normal condition conducting without a decrement. It is to be noted that in this case the response is approximately as large at the distal leads whether stimulation occurs at the proximal or distal stimulating electrodes (cf. 13, fig. 6). The only difference to be noted is that the duration of the response appears to be slightly greater when the impulse has travelled the longer distance. A probable explanation of this would seem to be that

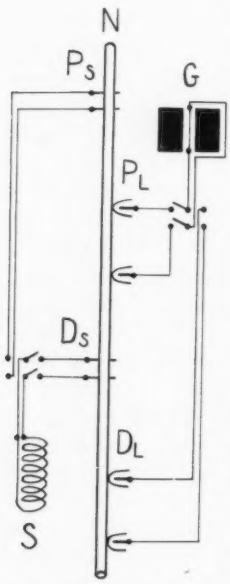

Fig. 7. Arrangement of electrodes on nerve to show decrement. *S*, secondary coil; *N*, nerve; *G* galvanometer; P_s, proximal stimulating electrodes; D_s, distal stimulating electrodes; P_l, proximal leads; D_l, distal leads.

the various fibers of the nerve trunk do not conduct with exactly the same velocity, and the time of arrival of the impulses in these different fibers is, therefore, less closely synchronous when they have travelled a long distance from the point of stimulation than when they have been set up by a stimulus much nearer to the leads (cf. 14). Of the seven nerves clearly shown to conduct with a decrement, some exhibited this condition when first removed from the body, but later recovered so that little or no decrement remained; others showed no conduction at all on removal from the body, but after a period of recovery in Ringer solution conducted with a demonstrable decrement.

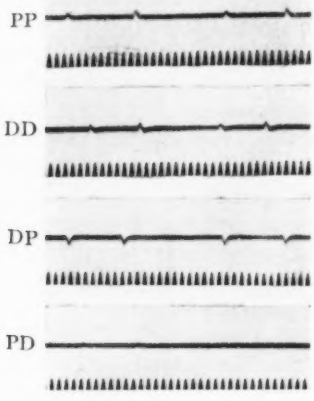

Fig. 8

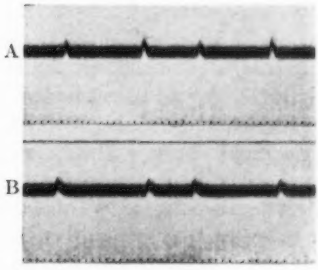

Fig. 9

Fig. 8. Action currents of cat's popliteal-tibial nerve, showing decrement. Nerve removed 3 hours and 20 minutes after death, kept in Ringer solution at room temperature for 1½ hours. Electrodes as in figure 7. Distance P_s to D_t about 10 cm. First letter designates stimulating electrodes used, second letter designates leads. Alternate make and break shocks; strength of break shocks in *PP*, *DD* and *DP*, 175 Z; in *PD*, 260 Z.

Cambridge galvanometer, 12,000-ohm string, magnification 300, tension 110 m. per amp.

Fig. 9. Monophasic action currents of normal popliteal-tibial nerve, showing absence of decrement. Nerve removed 23 minutes after death; records taken within 20 minutes. Two pairs of stimulating electrodes and one pair of leads: A, distance from point of stimulation to leads, 50 mm.; B, 110 mm. Break shocks 230 Z in both. Compare with *DD* and *PD* respectively in figure 8.

Hindle galvanometer; 17,000-ohm string; magnification 490; tension 104 m. per amp.

In one experiment with a nerve which had lain between dissected muscles in the living animal the same procedure was employed to determine whether in this case also conduction may occur with a decrement. The result agreed with that of the nerves which had lain in the dead body, decrement being clearly shown. In this respect, then, one more point of similarity appears between the two conditions.

The possible significance of the temporary loss of function. It is an interesting question what causes the temporary suppression of function which has been observed both in nerves lying undisturbed in the body after death and in nerves completely surrounded by the same tissues among which they normally lie, after these have been freely dissected in the living animal. Let us review briefly the main points of the evidence. Nerves removed from the body and placed in Ringer solution retain their functional capacity for a considerable time which is highly dependent on temperature. If the Ringer solution is maintained at body temperature the functional capacity of the nerve may disappear permanently in 2 or 3 hours. At ordinary room temperature, that is, about 20°C., function is usually retained from 4 to 20 hours; if the nerve is kept below 8°C. function is held in abeyance, that is, no electrical response results from stimulation, but if the nerve is warmed above 10° or 15°C. at any time up to 3 or 4 days after excision, the nerve may still manifest function. When an animal dies and the body is left undisturbed for 3 or 4 hours, a nerve removed after this time appears to be dead in that it shows no response to stimulation; but the loss of function is only temporary. Not only does the nerve recover in Ringer solution, but, if examined on the following day, it shows larger responses and survives longer than a similar nerve removed at death and kept constantly at the same temperature in Ringer solution. In short, while the function was held in abeyance the nerve was in reality to some extent protected from death. When a nerve in the living animal is dissected away from its blood supply and then enclosed in the wound in its natural position, completely surrounded by the dissected tissues there is a similar loss of function which also proves to be temporary. Nerves subjected to either treatment but not to the extent of complete abolition of function, conduct with a decrement,—a condition similar to that obtaining in nerves subjected to dilute alcohol.

What is it in the environment of a nerve in the body after death which produces this temporary loss of function and yet promotes the subsequent survival of the nerve? Amphibian nerves kept in an atmosphere

of pure nitrogen at first conduct with a decrement (15) and then cease to respond to stimulation, and function reappears when they are again given access to oxygen. The nerves of the cat's leg are largely surrounded by muscles. These muscles during life require considerable quantities of oxygen. When death occurs in a mammal the muscles soon lose their irritability, as a result of oxygen-want or of the accumulation of waste products, resulting from the cessation of circulation. Presumably, then, as soon as circulation ceases the muscles surrounding a nerve rapidly absorb from the blood and body fluids in the vicinity all the available oxygen. They also produce carbonic, lactic and other acids which may conceivably diffuse through the small intervening layers of connective tissue and come in contact with the nerve trunk. It is to be expected, then, that the environment of the nerve soon after death will become devoid of oxygen and may possess an appreciable concentration of acids.[2] The work of Mines (16) and of Cohn (17) shows that under these conditions a temporary loss of function occurs in diverse physiological processes. If in our case this were due to lactic acid poisoning, recovery could not occur with the nerve kept in a moist chamber from the moment of removal from the body and not placed in Ringer solution; for unless the nerve is bathed in fluid the lactic acid cannot possibly diffuse away from it unless (as is highly improbable) it is all converted first into carbon dioxid by oxidation. We have already mentioned that in two of the experiments in which loss of function occurred in nerves which had lain in the tissues after dissection in the living animal recovery was found to occur while the nerve was kept in a moist chamber, never having been in contact with Ringer solution. The same phenomenon was clearly shown in a nerve whose loss of function had occurred as it lay undisturbed in the body after death. The reappearance of function after both kinds of treatment under conditions such that lactic acid could not be washed away indicates that if lactic acid poisoning occurs at all it does not suffice to account for the loss of function. We may, therefore, conclude that the observed effects are due either to oxygen-lack or to excess of carbon dioxid.

A significant analogy to the effects found in these experiments is furnished by the experiment of Cohn (17) on the spermatozoa of the

[2] Since this was written, observations have been reported by Doctors Severinghaus, Koehler and Loevenhart at the meeting of the Biochemical Society, December, 1922, showing that in some mammalian tissues the post-mortem development of acidity is both marked and rapid, occurring in a very few minutes after death.

sea urchin. These were devoid of motility and remained alive for a very long time if the carbonic acid which they themselves produced was allowed to accumulate (or if another acid were added in suitable concentration). When the carbon dioxid was allowed to escape and the acidity to decrease, however, they at once became motile, but soon died. The degree of motility could be nicely regulated by adjusting the relative amounts of oxygen and carbon dioxid and with it the longevity, always in inverse ratio to the motility.

The dependence of the nerve trunk on oxygen has long been taken as evidence of its metabolism and this view has recently been reinforced by Tashiro's demonstration of carbon dioxid production by the nerve (18). The persistence of function in a nerve trunk bathed in Ringer solution is one among many bits of evidence of the remarkable economy of metabolism manifested by this tissue. The fact that function disappears in a nerve deprived of its blood supply and surrounded by muscles after death or by dissected and presumably damaged muscles during life, appears to indicate merely that this environment is one of oxygen-lack or of a correlated acidity. The fact that a nerve actually survives the longer for having lain 3 or 4 hours in the body after death suggests that when metabolism is stopped the materials which would be exhausted by metabolism are thereby conserved, as appears also to be the case when function is suspended by lowering the temperature to below 8°C. It is in this respect that the observations resemble those of Cohn on spermatozoa. On the other hand, the fact that a nerve left in the body as long as 19 hours after death is unable to recover its function, shows that there is a limit to this conservation. Whether this is due to the development of other injurious substances in the carcass or to the fact that the mere absence of oxygen in itself ultimately destroys the tissue, we are not in a position to say.

The fact that one nerve lying surrounded by dissected muscles in the living animal showed markedly better survival than the control nerve suggests that the same condition (oxygen-want or acidity or both) causing suppression of metabolism is in this case also the main cause of the loss of function. On the other hand, the difficulty in confirming this result, some nerves showing permanent loss of function after a comparatively brief time between the dissected muscles, suggests that probably another factor enters in. There may well be a form of poisoning resulting from contact with damaged tissues altogether distinct from what occurs in the body after death. In this connection the observations of Cannon (19) on the production of shock by sub-

stances taken into the blood stream from damaged muscles presents the possibility of interesting and important analogies, but on this question of the effect of contact with damaged living tissues our evidence is too scanty and the conditions too difficult of control to justify any definite conclusions. It is interesting to note that loss of function through lying surrounded by dissected muscles was more easily obtained in the decerebrate animal than in the animal under urethane anesthesia. Two possible explanations of this fact occur to us. One that there is a general lowering of metabolic activity in the tissues of the limb by urethane and a state of oxygen-want is thus less readily produced. The other is that blood pressure is usually lower after decerebration than in the intact animal under urethane. We have not enough quantitative observations to warrant a statement as to how generally this is the case, but in those animals in which measurements have been made we have usually found the decerebrate preparation to be characterized by a low blood pressure. Blood pressure falling below a certain critical level has been shown to interfere with the adequate supply of oxygen to the tissues (20, p. 15). Here again we have not sufficient facts to warrant any definite conclusions. In general the question of the effect of damaged tissues in the living animal upon nerve trunks must be left to future research.

The occurrence of conduction with a decrement as a result of conditions in the body probably involving exhaustion of oxygen and resulting increase of acidity, is of especial interest in connection with the appearance of a similar condition in the fatigued neuro-muscular junction and the likelihood that similar conditions occur in the fatigued synapse of the reflex arc. Adrian and Lucas showed that the fatigued neuro-muscular junction behaves like a region in a nerve trunk which, through the action of a narcotic, conducts with a decrement (21); they suggested that certain central synapses might owe their behavior to a similar condition. Adrian pointed out further that the fatigability of the reflex arc and its notable dependence on oxygen both suggest that conduction with a decrement in the synapse may well depend on acidity in its environment (22), (cf. also (23), pp. 373 and 375). It is, therefore, perhaps significant in this connection to find that when conditions exist in the body which favor increase in acidity a nerve trunk can be definitely shown to conduct with a decrement.

The effect of tension. In a considerable number of experiments in which nerves were dissected from the limbs of animals either during life or immediately after death, and every precaution was taken to avoid

drying or crushing, we were, nevertheless, unable to obtain electric responses. In these dissections the only possible source of injury seemed to be such stretching as occurred when the nerve was being dissected out from the surrounding connective tissues. We therefore decided to investigate by special experiments the effects of tension. For this purpose nerves were dissected out with great care to avoid the application of more tension than was absolutely necessary in removing them. In order to minimize tension very sharp scalpels and scissors were employed. Threads were tied tightly to the two ends of the excised nerve and at one end a loop was made in the thread in order that the hook of a spring balance could be readily inserted. The nerve was then placed on electrodes as usual in a moist chamber and after a series of action currents had been recorded with the galvanometer, it was removed, a spring balance was hooked into the loop, and the nerve was carefully stretched and held for about 1 second under the desired tension as indicated by the spring balance.

Eleven such experiments were performed on the sciatic nerve or its branches, peroneal or popliteal, in the cat. The tensions employed varied from 25 grams to 250 grams. In most experiments we started by applying a comparatively small tension and, after observing the effects for some time, removed the nerve again from the moist chamber and applied a greater tension. One nerve was stretched four different times.

The results of these experiments were not quantitatively uniform, but they all agreed in showing one thing, i.e., that surprisingly small tension applied for one second usually greatly reduces the size of the action current when the nerve is tested immediately afterwards. In most cases a considerable degree of recovery can be observed in the course of the next hour or so. Every time a tension of 25 grams or more was applied to a peroneal or popliteal nerve the action current showed a reduction in magnitude. One hundred grams or more applied either to peroneal or popliteal always sufficed either to abolish the action current or to reduce it to a small fraction of its normal size. There seemed to be considerable variation in the reduction caused by a given tension. One peroneal nerve in good condition whose action current caused a galvanometric excursion of approximately 12 mm. before stretching, was subjected to a tension of 100 grams. One minute later it showed no trace of action current. Twenty-five minutes later it still showed none and no further observations were made upon it. In contrast with this another peroneal nerve subjected to 50 grams showed

only a slight decrease in the size of response, and then when subjected to 80 grams showed a slight increase. This last must have been due to an accidental change in the position of the electrodes, for none of the other observations showed any such increase. In a more typical case 100 grams tension applied to the entire sciatic trunk caused appreciable reduction in the size of response, and 250 grams subsequently applied reduced it to less than a third of its original magnitude, but did not abolish it altogether. The results of a typical experiment on a popliteal-tibial nerve in good condition are shown in figure 10.

In this same experiment application of four pairs of electrodes (fig. 7) showed that the nerve, conducting with very little decrement before tension was applied, conducted with a marked decrement over 77 mm. after stretching with 25 grams; and after a tension of 50 grams the impulse failed to traverse this distance (see fig. 11).

For comparison three experiments were made upon the sciatic nerves of frogs. In one case a large bull frog was used and the nerve was stretched successively with 40 grams, 75 grams, 100 grams, 122 grams, 150 grams, and finally with 200 grams. The size of the electric response to a maximal stimulus remained approximately constant, showing no appreciable change after the application of any of these tensions. The experiment was repeated on two sciatic nerves from a common frog with essentially the same results. It should be noted that the nerves of the cat are very much larger than those of the frog. The average cat's peroneal has a cross section area amounting to about 2 sq. mm., the popliteal about 3 sq. mm., the sciatic trunk in the thigh, consisting of these two branches, therefore, having approximately 5 sq. mm. cross section. In contrast to this the sciatic nerves of the frogs used in the laboratory have a cross section of about 0.3 sq. mm. Assuming that the fibers in the frog and the cat do not differ greatly in size, this means that 100 grams tension applied to a frog's nerve amounts to about six times as much per fiber as it does when applied to a cat's peroneal, and fifteen times as much as when applied to a cat's sciatic nerve. The experiments show then that ten times as much tension per square millimeter cross section can be applied to a frog's nerve without impairing its response as that which, when applied to a cat's nerve, causes complete or almost complete disappearance of the response.

The marked effect of moderate tension in temporarily abolishing the functional response in a mammalian nerve trunk calls to mind the old-fashioned practice of stretching human nerves for the relief of neuralgia. In the years between 1877 and 1891 many statements appeared in the

literature of surgery concerning the effects, beneficial and otherwise, arising from stretching the sciatic and other nerve trunks in patients suffering from this disorder (24), (25), (26). Cases were reported in which relief or cure was alleged to have been effected by this procedure. It was stated that sensory impulses were stopped, but motor impulses not interfered with (25). Some authors maintained that paralysis of

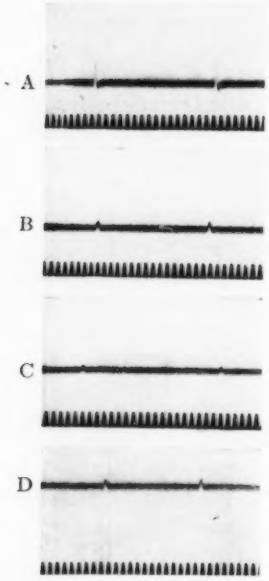

Fig. 10

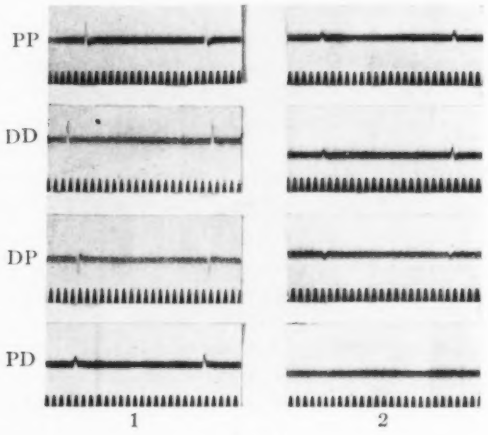

Fig. 11

Fig. 10. Action currents of popliteal-tibial nerve of cat, showing effect of tension applied to nerve. Distance from point of stimulation to proximal lead, 28 mm. Make and break shocks in each. A, before tension was applied, break shock, 146 Z; B, 2 minutes after stretching with 25 grams, break shock, 173 Z; C, 3 minutes later, 2 minutes after stretching with 50 grams, break shock, 173 Z; D, 1 hour later, break shock, 103 Z.

Hindle galvanometer; 25,000-ohm string; magnification 490; string tension 102 m. per amp.

Fig. 11. Conduction with decrement produced by tension. Same nerve as in figure 10. Lettering as in figure 8. Distance P_s to D_l about 100 mm. Make and break shocks in each. *1*, before tension was applied; break shocks, 146 Z. *2*, 2 minutes after stretching with 25 grams, break shocks, 173 Z.

Hindle galvanometer; 25,000-ohm string; magnification 490; string tension 102 m. per amp.

the muscles was liable to result. In the descriptions of the method employed it is stated that the hand should be passed under the sciatic nerve and it should then be pulled hard enough to lift the limb from the table. Elsewhere it is stated that to the sciatic nerve a tension of 18 or 20 kgm. can be applied, and elsewhere that it should not exceed 40 kgm.

The sciatic nerve trunk of the average-sized man has a cross section of approximately 30 sq. mm. It, therefore, requires 6 grams direct pull applied to the human sciatic to equal 1 gram applied to the cat's sciatic nerve in grams per square millimeter. Even allowing for this difference it is noticeable that the tension recommended by the surgeons in the literature cited as a therapeutic measure for human nerves amounted to more than twelve times as much per square millimeter cross section as that which sufficed in our experiments to abolish or nearly abolish function in the nerves of the cat. It is not surprising, therefore, that these measures should have produced a blocking of sensory impulses in the patients upon which they were employed. Our experiments on excised nerves have not been carried far enough to provide comprehensive data on the degree of recovery obtainable even in the absence of circulation. The end result when the blood supply is not disturbed, or, being disturbed, is later allowed to resume its connection with the nerve trunk, is something which our experiments do not touch; but the clinical records appear to indicate that in the motor nerve fibers the function may be restored so rapidly that no paralysis was discovered even in the case of the extreme tension applied in those days; on the other hand, they seem to show a considerably more prolonged effect upon sensory nerve fibers. It is open to question, however, whether these observations were sufficiently well controlled to warrant the final acceptance of this conclusion. In our experiments both afferent and efferent fibers combined to give the electric response to direct stimulation; therefore, we have not been able to discriminate between the two types of fiber as regards their behavior.

The great susceptibility of mammalian nerves to damage by stretching, which our experiments showed, doubtless explains a majority of the otherwise unaccountable failures of *freshly* dissected nerves to respond to stimulation, for considerable care is required to dissect a long nerve from the leg of the cat without subjecting it to a tension of more than 25 grams. Especially is this so in the case of the popliteal nerve, when its continuation as the tibial is followed through the deep tissues between the two halves of the gastrocnemius muscle. We have noticed

many more failures of this nerve to function than of the peroneal, which lies more superficially, and is, therefore, more easily dissected.

It is possible also that rapid drying may have been responsible for the disappearance of function in some cases. In several experiments nerves set up on electrodes in the open air in the usual atmospheric conditions of the laboratory have continued for many minutes to show good responses to stimulation, but, in one instance at least, a nerve set up in this way has shown a disappearance of function far more rapid than our previous experience had led us to expect, there being as yet no visible evidence of drying. When this rapid disappearance occurred the air may have been warmer and drier than usual. It may be that the increased osmotic pressure resulting from the early stages of drying caused impairment of function.

We are not satisfied that we have found and enumerated all the possible causes of failure to function in excised mammalian nerves. But we believe that if the precautions indicated by our experiments are carefully observed, mammalian nerves can usually be depended upon to respond normally to stimulation for a considerable time after excision.

SUMMARY

1. A mammalian nerve carefully dissected from the body during life or immediately after death, and kept in Ringer solution or in air in a moist chamber, retains an apparently normal functional capacity for a considerable time.

2. The time of survival is greatly influenced by temperature, becoming rapidly longer as the nerve is cooled until at temperatures below about 8°C. function is suspended and survival, that is, capacity to manifest function when warmed again, may last as long as 4 days.

3. When a mammalian nerve is left undisturbed in its normal relations in the body after death its function disappears much more rapidly than if placed in Ringer solution at the same temperature. But within limits this cessation resembles the suspension of function at temperatures below 8°C. in that if not left in the carcass too long the nerve recovers on removal to air or to Ringer solution, and then survives longer than the control nerve kept after the death of the animal in Ringer solution at the same temperature.

4. A mammalian nerve dissected from its blood supply and kept lying surrounded by dissected muscles in the living animal shows a similar loss of function which also is usually temporary. But the loss

of function under these conditions is not so regularly associated with a more prolonged subsequent survival than the control as it is in case of the nerve left in the body after death.

5. When a nerve is subjected to either of the above conditions but not for a long enough time to abolish function, it can often be shown to conduct with a decrement.

6. Probably in both of the above-mentioned conditions the main cause of the temporary loss of function is lack of oxygen, or accumulation of carbonic acid, or both. It may be inferred that prevention of metabolism promotes the conservation of something essential to survival and thus prolongs it. There is some evidence suggesting that there may be other toxic factors involved when a nerve is surrounded by damaged tissue in the living animal.

7. Mammalian nerves are exceedingly sensitive to stretching, such tension as may be accidentally imposed even during a fairly careful dissection of the nerve from the surrounding tissues sufficing often to reduce the electric response to a marked degree. Within limits there may be a recovery from this effect. In their susceptibility to tension mammalian nerves differ greatly from amphibian nerves, which, when subjected to many times the tension per square millimeter sufficing to reduce or abolish function in mammalian nerves, show no effect whatever.

We wish to thank Dr. C. C. Lund for assistance in some of the earlier experiments.

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STUDIES OF THE THYROID APPARATUS

X. The Refractive Index and Water Content of the Blood Serum of Male and Female Albino Rats Thyro-Parathyroidectomized and Parathyroidectomized at 100 Days of Age

FREDERICK S. HAMMETT

From the Wistar Institute of Anatomy and Biology, Philadelphia

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As a part of the general program of the study of the rôle of the thyroid and parathyroid glands in growth, the details of which have been given in a preceding paper of this series (1), it was believed that an investigation of the changes induced in the refractive index and water content of the blood serum by the removal of these glands might throw some light on the nature of the intermediary alterations following the loss of their function.

This seemed all the more probable in view of the experimental and clinical studies of Eppinger (2), which show a close relationship between thyroid activity and edema, and which have recently been confirmed in principle by Schaal (3). The results of the studies of the changes in the sera of thyroidectomized animals lack agreement. With regard to viscosity, Burton-Opitz (4) and Segale (5) reported a decrease, while Fano and Rossi (6), Paladino (7) and Gardella (8) found that an increase occurred. From the clinical side both Kottmann (9) and Deusch (10) obtained indications that the viscosity was above normal in myxedematous patients. The latter investigator advocates the determination of the refractive index, though with restrictions, as a diagnostic help in cases of myxedema and Basedow's disease. With regard to changes in the protein constituents of the serum, Ducceschi (11) in thyro-parathyroidectomized dogs observed an increase in the total proteins after the tetanic crisis, and a relative increase of the globulin with respect to the albumin. From measurements with the refractometer Deusch (10) concluded that the total protein of the sera of myxedematous individuals is increased. Such an interpretation should be accepted with reservations, however, in view of the findings of

Petersen, Jobling and Eggstein (12), that there is a progressive increase in the non-protein nitrogen and proteoses after the loss of the thyroid, and the indications of Levy (13) that the non-protein constituents of the blood increase under similar conditions; for it may be that the increase in refractive index found by Deusch is due to the increase in the non-protein refractive substances as well as to an increase in the protein constituents. As to whole blood, it has been reported by Kottmann (9), Levy (13) and Albert (14) that fibrin is increased after thyroid loss. Observations of the changes in the blood after parathyroidectomy which are pertinent to this study are few. Greenwald (15) found an increase in total phosphates in whole blood and serum and gives a report on one dog wherein a decrease in total solids and total nitrogen was observed.

An analysis of the observations which have just been reviewed fails to permit the prediction as to the nature of the changes to be found in refractive index and water content of the serum of thyroidless rats or other mammals. The results reported in this paper will show why this is so. In the case of the effects due to the loss of the parathyroid function a differentiation between the acute and the chronic condition is essential.

The sera used in this study were obtained from rats the growth of which has been reported in another place (1). The general details of the care and make-up of the six groups studied are given there and need not be repeated here. Suffice it to state that the rats were operated when 100 days of age and that the sera were taken at the time of dissection when the animals were 150 days old. The results then show the changes in the measured factors due to the chronic effects of glandular deprivation on sexually mature rats. Sex and litter controls were used in all cases. The animals were always dissected and the blood taken from 17 to 20 hours after the last feeding. The diet was the same for all. The methods used for getting and analyzing the serum were as follows: The rat was etherized in a jar from which it was removed while the heart was still beating. The heart was exposed, a cut made therein, and 2 or 3 cc. of the out-flowing blood collected in a small test-tube. The tube was stoppered and set aside for 1 or 2 minutes. The clot formed during the interval was broken up with a small rod; the tube was again stoppered and the whole centrifuged for 15 to 20 minutes. The resulting clear, light-yellow serum was quickly transferred with a pipette into the chamber of a Pulfrich-Zeiss refractometer and the refraction by the sodium flame measured. The

temperature of the chamber was taken, the chamber opened and the serum pipetted out into a weighing bottle, weighed and dried at 96 to 98° for 7 days for the per cent water.

The statistical data of the observed values corrected for 20°C. (16) are given in table 1. The individual observations are not tabulated but are on file at the Wistar Institute and are available for inspection.

Turning our attention first to an intergroup comparison of the males it is seen that the loss of the parathyroid function at the age of 100 days did not effect changes in the mean values of the refractive index or water per cent of the serum as compared with the non-operated controls.

TABLE 1

The statistical data for the refractive index and water content of the blood serum of the six groups of rats

ANIMALS		WATER			REFRACTIVE INDEX		
Group	Number	Mean	S.D.	P.E.M.	Mean	S.D.	P.E.M.
Males							
Controls	11	91.89	0.16	0.03	1.34829	0.00031	0.00006
Thypars	12	91.53	0.33	0.06	1.34897	0.00068	0.00013
Parathys	14	91.94	0.35	0.06	1.34820	0.00071	0.00013
Females							
Controls	11	91.92	0.47	0.10	1.34815	0.00086	0.00017
Thypars	13	92.08	0.56	0.10	1.34784	0.00133	0.00025
Parathys	15	91.99	0.46	0.08	1.34810	0.00083	0.00014

Thypars, Thyro-Parathyroidectomized rats.
Parathys, Parathyroidectomized rats.
S.D., Standard Deviation.
P.E.M., Probable Error of the Mean.

However, thyro-parathyroidectomy produced a decrease in water per cent and an increase in the refractive index. The bald values do not of themselves show whether or not the increased refractive index is a pure compensatory result of the decreased water content. Nevertheless, the conclusion is justified that the loss of thyroid function by sexually mature male rats 100 days of age causes an increase of the total solids of the blood serum. The findings as a whole check up the observations of Deusch (10) that the refractive index of myxedematous patients is higher than the normal and lends support to the results of other investigators with respect to the presence of an increased viscosity in thyroidless conditions.

As a matter of biological importance it should be noted that the variability of both factors of the operated groups is greater than that of the controls, a condition also found to exist in the body weight, body length and tail length values (1).

Turning now to the values for the three groups of females it is seen that neither of the operated groups differ, validly from the control group. Hence, the reaction of the females to the loss of the parathyroid function was the same as that of the males as far as the mean values are concerned. The loss of the thyroid function in the females apparently failed to produce a change in the serum concentration. Nevertheless, there is a hint of a tendency to a greater water content and a lesser refractive index, a result opposite to that obtained with the males. This paradoxical result is explicable, however, if there is taken into consideration the difference in the growth in body weight of the male and female thyroidless rats. At 100 days of age the mean weight of the male thypars was 160.0 grams and at 150 days it was 180.0 grams. At 100 days of age the female thypars had a mean weight of 140.8 grams, but, unable to regain and pass their initial weight during the 50-day interval as did the males, they weighed but 130.8 grams at 150 days. The female rats were then in a state of partial physiological inanition.

Now Polyani (17) found that starvation causes a decrease in the solids and refractive index of blood serum, while Jensen (18) reported that the serum concentration is reduced in fasting. Hence it is clear that the starvation effect produced in the females by the lack of the thyroid secretion not only balanced but tended to over-balance the desiccation effect on the serum observed in the thyroidless males.

One of the causes of the discrepancies in the reports of the changes in serum constitution after thyroidectomy is thus evident.

The observation of Dumitrescu-Mante (19) that the water content of the blood decreases as a result of an absolute fast does not discount this interpretation, since the female rats were not in a condition of absolute inanition but, as the records show (1), maintained the low weight obtaining after the loss of the thyroid at quite a constant level up to the time of dissection. Although Robertson (20) reported an increase in the total proteins of the serum of fasted rats he apparently considered the non-protein solids as constant and failed to record values for the water content. The two sets of observations are, therefore, not comparable on the basis of the mean values.

Comparing the males and females it is seen that there are no sex differences in the mean per cent of water or the mean refractive index either in the controls or the parathyroidectomized groups.

This analysis of the means of the observations of the six groups is insufficient, since it fails to allow a determination of whether or not the nature or distribution of the refractive substances of the serum has been altered by the lack of the glandular functions or shows a sex difference.

As a preliminary to such a determination certain ground work must be described. Hatai (21) has shown that there is a close relation existing between the refractive index of the serum of the albino rat and the age of the animal. With the help of Prof. F. H. Safford of the Department of Mathematics of the University of Pennsylvania, I have obtained an equation representing this relation and from which the curve designated as the age reference curve on the accompanying charts was plotted. The equation is of the general type, $x=k + a + by + cy^2 + dy^3 + ey^4$ and was derived from Hatai's observations of the refractive indices of the sera of rats, 37, 41, 44, 50, 52, 60, 64, 66, 72, 74, 80, 120 and 161 days of age. The result of equating these values gave:

$$x = 1.33320 + 0.002246 + 0.000448084y - 0.00000580504y^2 + 0.0000000349016y^3 - 0.000000000078454y^4.$$

where x is the refractive index and y the age. Since the observations of Hatai showed that certain irregularities in the general course of the increase in refractive index with increasing age occur around about the period of weaning and at puberty the selection of the observed data for the construction of the equation was guided accordingly. In table 2 the calculated values of the refractive index on age is given for the workable range. This is done because the equation is quite cumbrous for handling unless a calculating machine is available. Data from both sexes are combined. The curve obtained from these values is shown on the charts as the age-reference line 1.

It is a general law of the refraction of solutions that the index of refraction x is a linear function of the concentration of the solute. This law holds good for solutions of inorganic salts (22), for solutions of inorganic colloids (23), (24) and for organic colloid solutions (25), (26), (27). I have taken from Hatai's observations the observed values for the water content and refractive index of the serum for the ages of 10, 12, 37, 41, 50, 66, 69, 92, 120, 161 and 450 days and find that the law

holds good for the blood serum of the albino rat. Using the type equation $ax + by + c = °$ as the basis of the method of computation the equation derived from Hatai's values is $x = \frac{1 - 0.0012445y}{0.65607}$; where x is the refractive index and y the per cent water of the serum. The sum of the squares of the deviations of the observed from the calculated values is 0.0000000024. The slope of the equation is −0.5271. This line is shown on the charts as the water-refractive index or the second reference line.

TABLE 2

The tabulated values of the refractive index of the blood serum of the albino rat on age in days

AGE	N.D.	AGE	N.D.
34	1.34524	76	1.34867
36	1.34555	80	1.34880
38	1.34584	85	1.34893
40	1.34611	90	1.34905
42	1.34637	95	1.34916
44	1.34660	100	1.34926
46	1.34682	105	1.34936
48	1.34702	110	1.34946
50	1.34721	115	1.34956
52	1.34738	120	1.34967
54	1.34754	125	1.34977
56	1.34769	130	1.34986
58	1.34783	135	1.34995
60	1.34795	140	1.35003
63	1.34813	145	1.35009
66	1.34828	150	1.35012
69	1.34841	155	1.35012
72	1.34853	160	1.35007

These two equations make possible a comparison between observed and expected values for refractive index and water content on the basis of age, as will be shown presently.

Now it is obvious that all the rats used in this study were of the same calendar age. On the other hand, the physiological age as evidenced by growth of the various groups is obviously different. Hence, we can consider the groups from the point of view of their respective physiological ages as denoted by the attainment in size. As stated in the preceding paper (1), body weight growth is more variable and susceptible to disturbance than is growth in body length. Consequently,

body length is here taken as the criterion of physiological age. Using Donaldson's tables (28) it is possible to determine the physiological age of the rats of these groups in terms of the standard rat on the basis of the observed body length. This had been done and the values are given in table 3, together with the corresponding values of the refractive

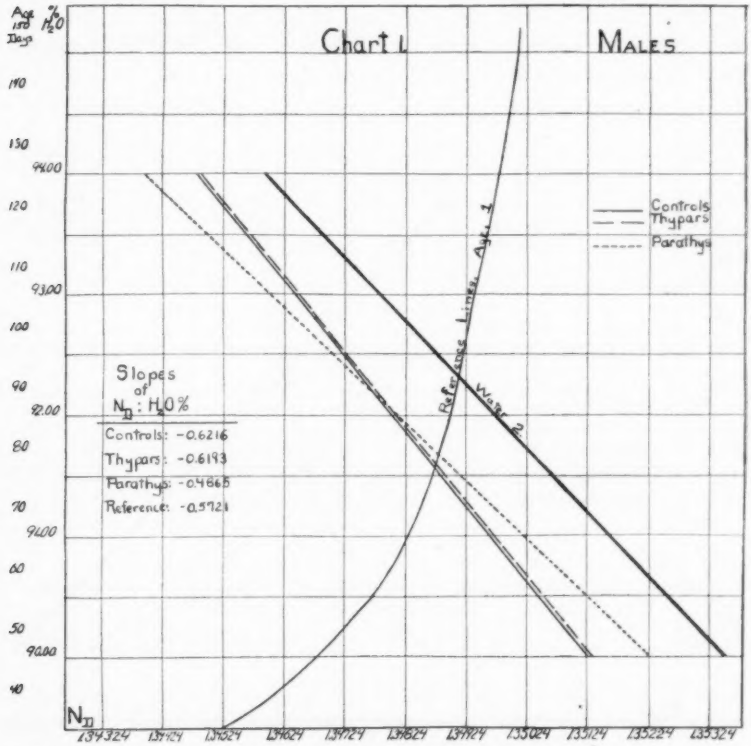

Chart 1. The reference lines and the lines plotted from the equations derived from the observations made on the control, thypar and parathy males.

indices taken from table 2. By substituting the calculated values for the refractive index in the equation for the water per cent-refractive index, there are obtained the values for the expected per cent of water of the sera at the various ages. This had been done and the results are given in table 3 together with the observed values for the per cent

water and the differences between the observed and the calculated values for both factors, and the observed body lengths.

A study of this table brings out the fact that the control rats of both sexes had the water per cent to be expected for the physiological age. On the other hand, the refractive index was low. This shows that the

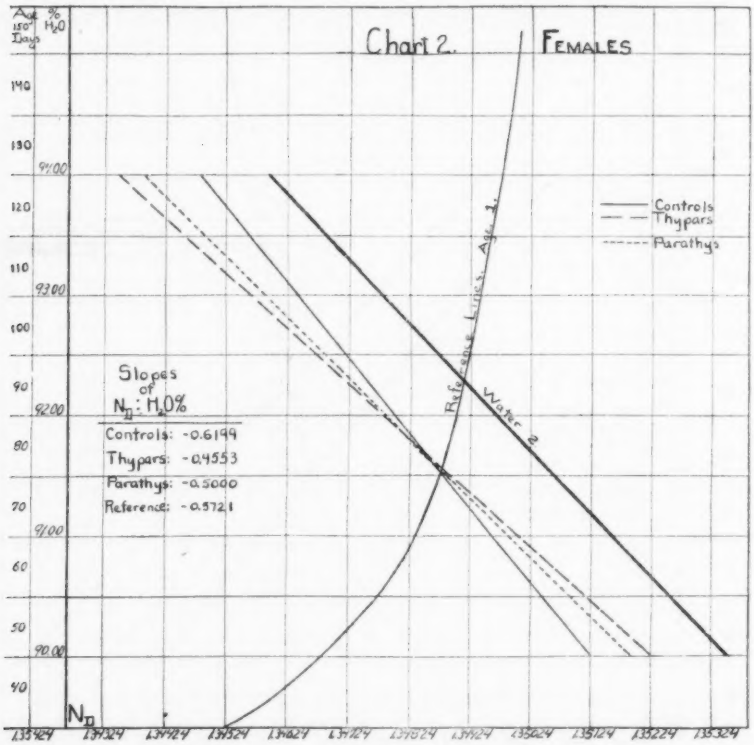

Chart 2. The reference lines and the lines plotted from the equations derived from the observations made on the control, thypar and parathy females.

nature or the distribution of the refractive substances aside from the solvent water, was different than that of the rats used by Hatai. From this it is evident that the determination of the refractive index of blood serum alone without a simultaneous determination of the per cent water is an invalid method for the estimation of the serum solids.

The water content of the sera of all the operated groups was lower than that to be expected on the basis of the physiological age. This is presumptive evidence that during the 50-day interval elapsing between the loss of the thyroid apparatus or the parathyroid glands and the time of the taking of the serum, the blood serum tended to become concentrated to a greater degree than it would have if the concentration due to an increasing age had followed the natural course as determined by growth in body length. Whether the relatively increased refractive indices of the operated groups is a purely compensatory reaction or not,

TABLE 3

Comparison of the observed values for refractive index and per cent water with the expected on the basis of physiological age determined from the body length measurements

	BODY LENGTH	AGE		REFRACTIVE INDEX			PER CENT WATER		
	Observed	Observed	Physiological	Observed	Calculated	Difference	Observed	Calculated	Difference
Males									
Controls	202.5	150	130	1.34829	1.34986	−0.00157	91.89	91.92	−0.03
Thypars	186.5	150	93	1.34897	1.34912	−0.00015	91.53	92.31	−0.78
Parathys	189.9	150	98	1.34820	1.34922	−0.00102	91.94	92.26	−0.25
Females									
Controls	190.2	150	133	1.34815	1.34991	−0.00176	91.92	91.90	−0.02
Thypars	176.0	150	92	1.34784	1.34909	−0.00125	92.08	92.33	−0.25
Parathys	177.5	150	96	1.34810	1.34918	−0.00108	91.99	92.28	−0.29

is not shown in this analysis. It should be noted that the greatest decrease in water per cent and the greatest relative increase in refractive index occurs in the male thypar group which substantiates the absolute value interpretation.

In order to determine whether or not the loss of the thyroid or parathyroid glands has caused a change in the nature or distribution of the refractive substances of the serum it is necessary to utilize the principle that the refractive index is a linear function of the concentration of the solutes. A comparison of the slopes and the positions of the lines representing this relation for the various groups as determined

from the equating of the individual observations of each group gives this information. The equations have been calculated and are given in table 4. The lines derived from the equations are plotted on the accompanying charts.

For convenience table 5 is given as the record of the values for the refractive index at water concentrations of 90.00 and 94.00 per cent.

Comparing first the male and female controls it is evident that since the lines of their equations have practically the same slope and the

TABLE 4

The equations of the linear relationship between the refractive index and the water content of the sera of the six groups

EQUATION	SLOPE	$\Sigma\Delta^2$
Males		
Controls $x = \frac{1 - 0.00107531y}{0.668435}$	−0.6216	0.0000000007
Thypars $x = \frac{1 - 0.0010778y}{0.668232}$	−0.6193	0.0000000134
Parathys $x = \frac{1 - 0.00135302y}{0.649451}$	−0.4865	0.0000000018
Females		
Controls $x = \frac{1 - 0.00107805y}{0.668244}$	−0.6199	0.0000000050
Thypars $x = \frac{1 - 0.00141718y}{0.645173}$	−0.4553	0.0000000023
Parathys $x = \frac{1 - 0.00130556y}{0.652780}$	−0.5000	0.0000000057

$\Sigma\Delta^2$—Sum of the squares of the deviations of the observed from the calculated.

same points of departure and arrival the sera of the two sexes do not differ from each other with respect to the nature or distribution of the refractive substances. Moreover, since they both have the same mean per cent of water and refractive index it can be concluded that there are no sex differences in the blood serum of the albino rat at 150 days of age.

Turning next to the thypar males it is found that the slope of their equation is practically the same as that of their controls and that

the position of the line constructed from the equation also differs but slightly from the latter. This taken together with the fact of the lowered mean water content and the increased refractive index of the serum as compared with the controls indicates, we believe, that the loss of the thyroid functions has produced a slight but significant desiccation of the blood serum without great alteration of the nature or distribution of the refractive solutes and that the increased mean refractive index is almost purely compensatory to the decrease in water content. This interpretation supports from a different experimental angle the hypothesis of Eppinger (2) that the thyroid gland is of importance in the fluid exchange.

TABLE 5

The values of the refractive indices of the sera of the various groups at designated water contents

	WATER	
	90.00 per cent	94.00 per cent
Males		
Controls	1.35125	1.34481
Thypars	1.35132	1.34487
Parathys	1.35226	1.34393
Females		
Controls	1.35127	1.34481
Thypars	1.35221	1.34349
Parathys	1.35191	1.34391

Considering now the sera of the parathyroidectomized rats it is seen that the slopes of the lines of the equations for both males and females are similar in nature and close in degree but yet are markedly different from those of their controls. That they cross the controls is to be expected since the means of the group values are practically the same. Such being the case it must be concluded that the loss of the parathyroid glands at 100 days of age does not change the concentration of the total solids of the blood serum of the albino rat as taken at 150 days of age but that there is produced a change in the nature or the distribution of the solids, which is probably in the same direction for both sexes.

The line of the equation of the female thypars clearly shows that here too there has been caused a change in the nature and distribution of the

refractive substances of the serum. This falls in line with our interpretation of the difference in response to thyroid loss of the males and females as based on the findings of Polyani (17) and Jensen (18) in inanition.

This new method of analysis demonstrates the insufficiency of the evidence obtainable from a single determination of the refractive index of blood serum with or without a simultaneous determination of the water per cent, in giving an indication of whether or not a change has occurred in the nature or distribution of the serum solids following a given experimental procedure. Although the formula of Robertson (29) for the determination of the constant of refraction for a given substance in solution is valid for solutions of known concentrations of constituents, it is quite inadequate for a system such as the blood, where changes in water per cent may induce changes in the absolute or relative concentrations of the various solutes due to the variable permeability of the vascular structures.

The method of analysis developed in this study fails to yield information as to the extent or type of change in the nature or distribution of the refractive substances. It does, however, show whether or not a change has occurred.

SUMMARY AND CONCLUSIONS

The refractive index and the water per cent of the blood serum of male and female control, thyro-parathyroidectomized and parathyroidectomized albino rats has been determined.

By a new method of analysis of the observations described in the text it was found that:

1. There is no sex difference in the water content or nature or distribution of the refractive substances of the serum in normal rats, 150 days old.

2. The loss of the parathyroid glands by sexually mature male or female animals 100 days of age does not cause any alteration in the per cent of total solids of the blood serum which appears when the rats are 150 days old. This loss, however, does cause a change in the nature or the distribution of the refractive substances aside from the solvent water. This change is in the same direction and of approximately the same degree in the two sexes.

3. The loss of the thyroid gland by male rats of the same absolute age causes a decrease in the water content of the serum and an increase in the refractive index of such a nature as to allow the conclusion that

a true partial desiccation of the serum has occurred. This is supportive evidence that the thyroid gland is concerned in the fluid exchange of the body.

4. The loss of the thyroid gland by the females of the same absolute age, produces a state of partial inanition, the effects of which on the serum (e.g., tendency to decreased concentration) counter-balance the desiccatory effect of the thyroid loss observed when anabolism and growth in body weight occurs after such a loss.

5. When the observed values for the per cent water are compared with the calculated values on the basis of the physiological age on body length and the reference equations, it is seen that the controls of both sexes have the water concentration to be expected, while all of the operated groups show a tendency toward a slightly lower per cent of water than would be expected.

6. The determination of the refractive index of blood serum without a simultaneous estimation of the water per cent is insufficient as a measure of the serum solids.

7. In a system such as the blood, where changes in water content may mean changes in absolute and relative concentrations of the serum constituents due to changes in vascular permeability or filtration, the determination of the refractive index and water per cent in a single sample of blood is insufficient to show whether or not the nature and distribution of the serum solids has been changed.

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THE COLLOID CHEMISTRY OF PROTOPLASM

I. General Considerations
II. The Electrical Charges of Protoplasm

L. V. HEILBRUNN

From the Department of Zoölogy, University of Michigan, Ann Arbor

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I. General considerations. In recent years the subject of colloid chemistry has advanced steadily. Books like those of Zsigmondy[1] contain a great mass of data which is apparently capable of intelligent organization. True, one or two workers have attempted to shake the foundations of the subject, but this attack, although pushed vigorously, has never attempted to take into account the fundamental facts around which the subject of colloid chemistry has been built up.

The colloid chemist uses a variety of methods in this work, but these may be more or less artificially divided into two groups. Generally speaking, he may either observe the various physical properties of colloids or he may determine the effect of chemical or physical forces on these properties. The second side of the subject is more truly experimental. After treatment with this or that reagent, the colloid chemist notes changes in general appearance, changes in osmotic pressure or electric charge, or changes in viscosity. Colloidal systems often vary their viscosity rapidly and these variations may be very marked. It is for this reason that viscosity changes are perhaps the best indicators of colloidal processes. This is especially true for the student of the colloidal properties of protoplasm. In a test-tube one can readily observe coagulation or gelation. In a living cell the ordinary microscope gives no certain information concerning such changes and some sort of a viscosity measurement is necessary for a decision.

Protoplasmic viscosity can be measured. The centrifuge method is capable of furnishing data which are certainly trustworthy.[2] This method has made possible a comprehensive and thorough study of the

[1] R. Zsigmondy, Kolloidchemie, 3te Auflage, Leipzig, 1920.

[2] L. V. Heilbrunn, 1921, Journ. Exper. Zoöl., xxxiv, 417.

colloid chemistry of protoplasm. The biologist can now try out the effects of various reagents and treatments upon the colloidal behavior of protoplasm, for viscosity tests provide a convenient index of changes in colloidal condition.

It is of course true that in measuring the viscosity of protoplasm, it is not possible to employ as rapid and accurate tests as those which may be used on colloids in mass. Students of physics and chemistry must remember that the biologist is under a very severe handicap when he attempts to apply physico-chemical methods to the living cell. Oftentimes he is unable to make such exact measurements as the physicist considers essential, and he must be content with whatever approximate tests are at all possible. This is a direct consequence of the peculiar nature of living material.

As a colloidal system protoplasm is extremely complex. The cytologist and the biochemist in their study of protoplasm have accumulated a certain amount of morphological and chemical information which indicates a high degree of complexity.

Chemically protoplasm is known to consist of a variety of substances: proteins, fats and lipoids, carbohydrates and inorganic salts. Cytological study has helped to locate these substances within the cell. It is generally admitted that protoplasm is a watery solution which contains dissolved in it salts and perhaps carbohydrates. The proteins are in a state of colloidal solution, but not completely so, for many of the granules found in protoplasm are doubtless protein and these are in suspension. The fats and lipoids are usually emulsified and are scattered through the cell as tiny droplets which apparently constitute most of the mitochondria. Thus protoplasm is at one and the same time a true solution, a colloidal solution, a suspension and an emulsion.

Physically, few facts are known concerning protoplasm. Usually it is quite fluid and now and then it can be observed to flow. At times, however, this fluid condition ceases, the protoplasm becomes stiff and gel-like. Such changes are just beginning to be known. Probably they occur widely and they are doubtless of great significance in many forms of vital activity.

If protoplasm is a watery liquid it must be prevented in some way from diffusing into the watery liquids which in every case surround it. Such a diffusion is prevented by the organization of protoplasm into cells. Each watery droplet of protoplasm must of course be surrounded by some sort of a membrane. Concerning this membrane many conflicting ideas, based largely if not wholly on theory, have occupied a

considerable space in the literature. In many animal cells this membrane is clearly visible and it can be studied directly. Such a study has been made in the case of certain egg cells and the evidence shows the membrane to be essentially a protein gel.[3]

Obviously the student of the colloid chemistry of protoplasm faces no easy problem. Difficulties are only too numerous. But there is one encouraging side to the problem. The protoplasm of all cells, both plant and animal, is apparently very much alike. The morphologists emphasized this point many years ago. Biochemical research some time later showed that the essentiai substances of living organisms are much the same in the most varied types of plant and animal life. It is believed that the colloidal behavior of protoplasm is if anything even more uniform. Thus for example almost all living things are killed at a temperature of from 30° to 45° and this death is probably a direct result of coagulation.[4] It is probable that every type of protoplasm will be found to have very nearly the same coagulation temperature. Other examples of constancy in the colloidal behavior of protoplasm will appear in the course of this study.

II. The electrical charges of protoplasm. Perhaps the most important characteristic of a colloid is its electric charge. It is this charge that keeps the colloidal particles separate, and the subdivision of matter in colloidal form is largely an electrical phenomenon. All colloidal solutions have particles charged either positively or negatively and the behavior of the colloid is primarily determined by the nature of the charge.

The recent books on colloid chemistry emphasize more and more the importance of these electrical phenomena. In a study of the colloid chemistry of protoplasm it is essential to determine whether the colloids which compose it carry a positive or a negative charge. This knowledge is a necessary prerequisite to any understanding of protoplasmic dynamics, a fact which was pointed out as long ago as 1905 by the physical chemist Billitzer.[5]

So far no one has systematically endeavored to solve the problem. Some years ago Barratt[6] attempted to discover whether the protoplasm of Protozoa was acid or alkaline. In an acid solution, such a colloid as protoplasm would probably bear a positive charge owing to the

[3] L. V. Heilbrunn, 1915, Biol. Bull., xxix, 149; 1920, Biol. Bull., xxxviii, 317.
[4] Evidence to be presented later.
[5] J. Billitzer, 1905, Zeitschr. f. physik. Chem., li, 128.
[6] J. O. W. Barratt, 1905, Zeitschr. f. Allg. Physiol., iv, 87.

excess of hydrogen ions, but this would not necessarily always be true.[7] Similarly, in alkaline solution the colloidal particles of protoplasm would probably bear a negative charge. However, Barratt was unable to decide whether protoplasm was acid or alkaline, for he found that the indicators he used could not be trusted.

In order to determine the electric charge of a colloid, the colloid chemist uses various methods. The most widely used method is to observe the direction in which colloidal particles migrate under the influence of an electric current. If they go to the cathode they are positively charged, if to the anode negatively charged. The migration of living cells under the influence of an electric current can also be studied. But this method when applied to the living cell is an index largely of the charge at the surface of the cell; it gives no definite information concerning the electric charges within protoplasm.

A second method of determining the electric charge of a colloid may be termed the precipitation method. According to the usual theory, salts precipitate colloids by neutralizing their electric charges. This neutralization is accomplished by the adsorption of ions possessing an electric charge opposite in sign to that on the colloidal particles. It is known that some ions are adsorbed more readily than others. Usually, though not always, ions of higher valence are the more readily adsorbed. If, when a colloid is treated with various salt solutions, it is found that the salts with more readily adsorbed cations are more effective as precipitants, this indicates a negative charge on the particles of the colloid. Such salts with readily adsorbed cations would protect rather than precipitate a colloid charged positively. Here then is a method of test. It is a standard method and for this reason the principles underlying it have been discussed in a rather summary fashion. A fuller discussion can be found in various books on colloid chemistry (cf. Bancroft l.c. pp. 212–224).

Bancroft[8] gives the order of adsorption of cations by egg albumen as

$$Th > UO_2 > Cu,Zn > Ca > Mg > Li > K,Na > NH_4$$

Of course, the order of adsorption of ions by protoplasm is not known but it is probably not very different from that which holds for egg albumen. At any rate the general order of the series is presumably

[7] Due to selective adsorption. The hydrogen ion concentration of the dispersion medium is not a measure of the adsorbed hydrogen ions, see W. D. Bancroft, *Applied Colloid Chemistry*, New York, 1921, p. 211.

[8] W. D. Bancroft, l.c., also 1915, Journ. Phys. Chem., xix, 349.

similar. If then we treat protoplasm with solutions of various salts and we find that calcium and magnesium salts precipitate more readily than sodium or potassium salts, we would conclude that protoplasm is negatively charged. If on the other hand the reverse were found to be true, we would conclude that the colloidal particles of protoplasm bore a positive charge. By comparing the action of various salts on protoplasm we can obtain evidence as to the nature of its electric charges.

In studying the effect of salts on protoplasm, morphological observation is not sufficient. Merely by looking at protoplasm we can not decide whether it has become coagulated or not. Some sort of a physical test is essential. When a colloid gels its viscosity increases greatly, it becomes rigid. When protoplasm gels or coagulates a marked rise in viscosity occurs. Any test of protoplasmic viscosity therefore provides an index of coagulative change. The various methods for making protoplasmic viscosity determinations are discussed in a recent paper.[9] In the experimental work discussed in this paper, the centrifuge method was used exclusively.

It is quite a simple matter to test the effect of various salts on the protoplasm of sea-urchin eggs. These eggs have often been used in cytological studies; they are easy to obtain and their size of about 80 microns makes them convenient to work with. The experimental work on sea-urchin eggs was done during the summer of 1921 at the Woods Hole Marine Biological Laboratory. The experiments were repeated in 1922, but with one exception only the 1921 results will be reported. The later results are merely confirmatory. Sea-urchin eggs were placed in solutions of various chlorides and then after a certain time interval, the viscosity of their protoplasm was compared. The viscosity measurements were not as rigidly quantitative as it might by much longer study be possible to make them. The present research did not attempt to measure the exact effect of the various salts, its main purpose was to discover the nature of the electric charges of protoplasm. To settle this question only comparative data are necessary.

In studying the effects of salts on sea-urchin eggs two precautions must be taken. The salt solutions used must be isotonic or approximately isotonic with sea-water. Secondly, they must not show marked changes in hydrogen ion concentration.

[9] Heilbrunn, 1921, Journ. Exper. Zoöl., xxxiv, 417.

It is easy enough to obtain isotonic solutions of the salts to be employed. Sea-urchin eggs are good osmometers, they swell in hypotonic solutions and shrink in hypertonic solutions. By testing out a range of dilutions, it is possible to select the one concentration which neither causes the eggs to swell nor shrink. This may be regarded as isotonic.

Secondly, it is generally possible to guard against changes in hydrogen ion concentration by using instead of pure salt solutions mixtures of salt solutions and sea-water. In the mixtures used, the hydrogen ion concentration was scarcely different from that of sea-water. Some experiments on the effect of hydrogen ion concentration on the viscosity of sea-urchin egg protoplasm indicate that the pH may vary between 7.0 and 9.0 without producing any immediate effect.

It might be thought that different salts and ions would enter protoplasm at different rates of speed, and that this variable permeability would render our experiment indecisive. No doubt slight differences in permeability occur. It was found however that all of the salts used entered the cell quite rapidly, for all of them produced viscosity changes in a comparatively short time. The experimental facts do not fit in with any interpretation based on permeability. A difference in permeability might perhaps account for the fact that some salts produce coagulation more rapidly than others, but it could in no way explain why some salts increase protoplasmic viscosity and others decrease it. And this is precisely what was found to occur.

The chlorides of calcium, magnesium, potassium, sodium and ammonium were used. Let us consider a sample experiment. Sea-urchin eggs were placed in isotonic solutions of sea-water plus sodium chloride, and sea-water plus magnesium chloride. In order to determine the isotonic concentration of magnesium chloride various dilutions were tried. A 0.3 M magnesium chloride solution[10] is approximately isotonic, eggs placed in such a solution shrink only very slightly As is well known, a 0.6 M NaCl solution is also slightly hypertonic.

In the following description of our experiment and in subsequent descriptions of the same sort, certain abbreviated forms of expression were used. Centrifuge tests were in every case made with a two speed hand centrifuge of the Bausch and Lomb type.[11] All tests were made

[10] All salt solutions were made up by weight and were not titrated. The concentrations given are therefore not very exact.

[11] In this centrifuge, the distance between the ends of the tubes (hematocrit attachment) is about 12 cm. One turn of the high speed handle produces 130 turns of the tubes.

with the high speed handle. In designating the centrifugal treatment given to the eggs, a simplified form of expression was employed. This may be illustrated by an example. The statement "H.S. 15 in 15" would indicate that the high speed handle was turned 15 times in 15 seconds at a uniform rate of speed. When eggs are so treated the granules in the cytoplasm move in a centrifugal direction and the lipoid or oil droplets move in a centripetal direction. As a result four zones appear in sea-urchin eggs (see earlier papers on the subject; e.g., Lyon[12]). There is a "gray cap" which contains oil, a "hyaline zone," a "granular zone" and a "pigment zone." The distinctness with which these zones appear is an index of the fluidity of the cytoplasm. The width of the hyaline zone is perhaps a more accurate index. This is roughly estimated in terms of the fraction of the egg diameter included in the zone. Thus "H.Z. $\frac{1}{3}$" signifies that the hyaline zone extends over about one-third of that diameter of the egg which is perpendicular to the zones.

July 30. At 10:08 a.m., sea-urchin eggs were placed into two Stender dishes, A and B. Of these A contained 20 cc. 0.3 M $MgCl_2$ + 10 cc. sea-water, whereas B contained 16 cc. 0.6 M NaCl + 8 cc. sea-water. At 10:23 a.m., eggs from A and B were centrifuged simultaneously (in separate tubes of course), H.S. 20 in 20. In the eggs from A 4 zones showed plainly and H.Z. was $\frac{1}{3}$. In eggs from B no distinct zones appeared and the hyaline zone was barely indicated. Thus a sharp difference appears within 15 minutes.

At 10:41 eggs from A and normal control eggs which had been lying in sea-water were centrifuged simultaneously, H.S. 20 in 20. Not much difference could be observed. However when eggs from A and normal eggs from sea-water were centrifuged at 10:58 a.m. H.S. 35 in 38, a difference could be noted. In the eggs from A, 4 zones showed beautifully, the H.Z. was $\frac{1}{3}$–$\frac{1}{2}$ and the pigment zone was sharply marked off from the granular zone. On the other hand the normal eggs in sea-water had H.Z. $\frac{1}{4}$ and not very clear, their pigment zone was not sharply separated from the hyaline zone.

This experiment shows that magnesium chloride in isotonic concentration tends to liquefy the protoplasm of sea-urchin eggs. Sodium chloride in isotonic concentration has no such action and experiments both in 1921 and 1922 indicated that it tends to coagulate rather than liquefy.

The liquefying effect of calcium chloride is more marked than that of magnesium chloride. Measurements of egg diameters in various dilutions of calcium chloride showed the isotonic concentrations to be

[12] E. P. Lyon, 1907, Arch. f. Entwicklungsmech., xxiii, 151.

approximately 0.3 molecular. Such a solution causes a very slight shrinkage. It is easy to detect a difference in the action of magnesium and sodium chlorides, but the difference between calcium and magnesium is not so great and a larger number of tests is necessary in this case.

August 19. Two series of Stender dishes were used; of these A-F contained calcium, A′-F′ magnesium chloride.

A contained 20 cc. sea-water 5 cc. 0.3 M $CaCl_2$
B contained 20 cc. sea-water 10 cc. 0.3 M $CaCl_2$
C contained 20 cc. sea-water 15 cc. 0.3 M $CaCl_2$
D contained 10 cc. sea-water 10 cc. 0.3 M $CaCl_2$
E contained 10 cc. sea-water 15 cc. 0.3 M $CaCl_2$
F contained 5 cc. sea-water 10 cc. 0.3 M $CaCl_2$

In A′, B′, C′, D′, E′, F′, the concentrations were in every case the same as those in A, B, C, D, E, F, respectively, except that 0.3 M $MgCl_2$ was substituted for 0.3 M $CaCl_2$. Sea-urchin eggs were placed in A, B, C, D, E, F and A′, B′, C′, D′, E′, F′ at 12:35 p.m. At 1:07 p.m. the cytoplasmic viscosity of eggs from D and from D′ was compared, the centrifugal treatment was H.S. 25 in 25. In eggs from D, H.Z. was greater than $\frac{1}{4}$, but less than $\frac{1}{3}$. In eggs from D′ it was $\frac{1}{4}$, thus apparently a trifle less than in eggs from D.

At 1:17 p.m. eggs from B and B′ were centrifuged simultaneously, H.S. 25 in 25. Eggs from B had H.Z. greater than $\frac{1}{3}$, but less than $\frac{1}{2}$, eggs from B′ had hyaline zone $\frac{1}{3}$.

At 1:27 p.m. eggs from A and A′ were centrifuged simultaneously, H.S. 25 in 25. Eggs from A had H.Z. nearly $\frac{1}{2}$, eggs from A′ had H.Z. $\frac{1}{3}$.

At 1:39 p.m. eggs from E and E′ were centrifuged simultaneously, H.S. 25 in 32. Eggs from E had H.Z. $\frac{1}{3}$, those from E′ had H.Z. $\frac{1}{4}$.

At 1:49 p.m. eggs from C and normal eggs in sea-water were centrifuged simultaneously, H.S. 25 in 25. The eggs from C showed H.Z. greater than $\frac{1}{3}$ and nearly $\frac{1}{2}$, their pigment zone was separated from the granular zone though not completely. The normal eggs in sea-water had H.Z. $\frac{1}{3}$, their pigment zone was not separated from the granular zone.

In the above experiments the temperature of both air and water was 26° (at 1:45 p.m.). Measurements of hydrogen ion concentration were made with a phenolsulphonephthalein indicator set at 1:55 to 2:00 p.m. The pH of the solution in C was 7.9, in D 7.9, in E 7.8, in F 7.7 or 7.8, in E′ 7.7, in F′ 7.5.

The experiment shows that calcium chloride exerts a somewhat more marked liquefying effect than magnesium chloride. Or, stated in other words, magnesium chloride has the greater coagulative action.

In comparing the action of potassium chloride and sodium chloride, it was found that the former did not cause as much coagulation as the latter.

June 18. At 10:25 a.m. eggs were placed into two Stender dishes A and B, of which A contained 20 cc. sea-water + 20 cc. 0.6 M NaCl and B contained 20 cc. sea-water + 20 cc. 0.6 M KCl. At 10:45 a.m. eggs from A and B were centrifuged simultaneously, H.S. 35 in 35. The eggs from A had a hyaline zone but it did not show distinctly, whereas the eggs from B had H.Z. ½ and the H.Z. was clear and distinct.

Ammonium chloride is a more powerful coagulant than sodium chloride.

August 8. At 3:35 p.m. eggs were placed into two Stender dishes A and B, of which A contained 24 cc. 0.55 M NH_4Cl + 12 cc. sea-water; B contained 25 cc. 0.55 M NaCl + 12½ cc. sea-water. It had previously been shown that 0.55 M NH_4Cl was approximately isotonic for sea-urchin eggs. At 4:23 p.m. eggs from A and B were centrifuged simultaneously, H.S. 45 in 45. The eggs from A failed to show a distinct hyaline zone, the presence of a zone was indicated but that was all. The eggs from B showed a sharply defined hyaline zone, it was ½.

Potassium chloride exerts a stronger coagulative action than magnesium chloride.

August 3, 1922. At 10:00 a.m. eggs were placed into Stender dishes A and B. A contained 10 cc. sea-water + 20 cc. 0.5 M KCl,[13] B contained 10 cc. sea-water + 20 cc. 0.3 M $MgCl_2$. At 11:28 a.m. eggs from A and B were centrifuged simultaneously,[14] H.S. 30 in 30. The eggs from A had H.Z. ½ and there was no sharp distinction between granular and pigment zones. The eggs from B had H.Z. ½ and their granular zone was sharply marked off from the pigment zone.

The experiments described in the preceding pages are representative ones, and they are supported by other experiments of the same sort. They show clearly that the bivalent cations calcium and magnesium do not exert as powerful a coagulative action as the monovalent cations sodium, potassium and ammonium. On arranging the cations in the order of their coagulative action the following series is obtained:

$$Ca < Mg < K < Na < NH_4$$

The results obtained for sea-urchin eggs might be thought peculiar to this type of protoplasm. Further experiments were therefore tried on the protoplasm of Protozoa. Such experiments are not as satisfactory as those with marine eggs. In egg cells as a result of centrifugal

[13] This concentration of KCl is slightly hypotonic. In view of the fact that hypotonicity tends to produce liquefaction (Heilbrunn, 1920, Journ. Exper. Zoöl., xxx, 211), this fact does not detract from the value of the experiment.

[14] In this experiment a new model Bausch and Lomb centrifuge was used. The distance between the ends of the tubes is 14.6 cm., and one turn of the high speed handle produces 130 turns of the tubes.

treatment, three or four zones are characteristically formed; in Protozoa such extensive zone formation usually does not occur. When Stentor is centrifuged the granules move to one side of the cell, leaving the rest of the cell more or less clear. It is possible to compare the amount of granule shifting in Stentor under various conditions, and a comparative measure of the protoplasmic viscosity may be obtained. The method is admittedly crude, but it is a decided advance over the guesses that have been made in the past by those who have attempted to determine the action of salts on the protoplasmic consistency of Protozoa (Greeley, Spek[15]).

Another disadvantage of protozoan material lies in the fact that it is rather difficult to decide upon what constitutes an isotonic solution. The difficulty is not a very serious one for if one chooses concentrations dilute enough, the amount of osmotic interchange is certainly small. One can not be sure that a given concentration of a salt like calcium chloride is equal osmotically to some other concentration of sodium chloride. The theoretical osmotic pressure of the solutions is by no means a perfect index of the pressure they exert against the protozoan plasma membrane. For certainly the plasma membrane is not a perfect semipermeable membrane, and if calcium and sodium chlorides differ in the ease with which they penetrate the membrane, this introduces a factor which can not be reckoned with. The difficulty can to some extent be avoided by using solutions which, if not equal in their osmotic effects, are unequal in such a way that any osmotic differences would favor viscosity differences of the opposite sort from those obtained in the experiment. Thus, if we expect to find that calcium chloride exerts less of a coagulant action than sodium chloride, we use a solution of calcium chloride which we would expect to be osmotically stronger than the sodium chloride solution we compare it with.

In very dilute solutions of electrolytes like sodium chloride or calcium chloride, we can assume complete or practically complete dissociation into ions. Assuming a perfect semipermeable membrane, a solution of calcium chloride, since it dissociates into three ions, would have one and a half times the osmotic pressure of a solution of sodium chloride of the same molecular concentration. Thus for example an $\frac{m}{48}$ calcium chloride solution would equal osmotically an $\frac{m}{32}$ sodium chloride solution. In sea-urchin eggs sodium chloride coagulates the proto-

[15] A. W. Greeley, 1904, Biol. Bull., vii, 3; J. Spek, 1921, Acta Zoölogica, 153.

plasm more readily than calcium chloride; we might expect the same relation to hold for Protozoa if we were to compare an $\frac{m}{32}$ sodium chloride solution with an $\frac{m}{48}$ calcium chloride solution. It is possible that such a calcium chloride solution is weaker osmotically than the sodium chloride solution. In the experiments some allowance was made for this possibility, and a somewhat stronger solution of calcium chloride was employed. The concentration actually used was $\frac{m}{40}$. As a matter of fact, in practically all of the experiments in which chlorides of bivalent cations were compared with chlorides of monovalent cations, an $\frac{m}{40}$ solution was used for the former and an $\frac{m}{32}$ solution for the latter.

In making up the solutions especial care was taken to use pure water. Ordinary distilled water was first redistilled in a fused silica distilling apparatus.[16] The pH of this redistilled water was approximately 6.6. In general the solutions made with it were more alkaline than this, the pH of the NH_4Cl, LiCl solutions being about 6.8, and that of the others about 8.0. Experiments were made on Stentor coeruleus. The centrifuge used in these experiments was a Bausch and Lomb two speed hand centrifuge, new model.[17]

November 9. At 10:12 a.m. some Stentors were placed into dishes A and B. A contained 6 cc. $\frac{M}{32}$ NaCl and B, 6 cc. $\frac{M}{40}$ $CaCl_2$. At 10:39 a.m. Stentors from A and B were centrifuged simultaneously, H.S. 75 in 73. No difference could be detected. At 12:59 p.m. Stentors from A and B were centrifuged again, H.S. 75 in 75. As a result of this treatment, the Stentors from A showed no movement of granules, whereas those from B had their granules thrown over to one side of the cell.

November 14. At 1:33 p.m. Stentors were placed into A and B. A contained 6 cc. $\frac{M}{32}$ NaCl and B, 6 cc. $\frac{M}{40}$ Mg Cl_2. At 3:20 p.m. Stentors from A and B were centrifuged simultaneously, H.S. 75 in 75. In Stentors from B, granules were shifted to one side as a result of the centrifugal treatment, and dark and clear zones were sharply marked off. In Stentors from A there was little or no shifting of granules. The Stentors from B remained motile, those from A had ceased their movements.

These experiments indicate that sodium chloride exerts a coagulative action on Stentor protoplasm, whereas calcium and magnesium do

[16] Kendall conductivity still.

[17] See footnote 14.

not. This action is not confined to sodium chloride but is also exerted by the chlorides of potassium, lithium, and ammonium.

November 15. At 4:28–4:30 p.m., some Stentors were placed into A and B. A contained 6 cc. $\frac{M}{32}$ LiCl and B contained 6 cc. $\frac{M}{40}$ $CaCl_2$. The Stentors in A and B were centrifuged simultaneously at 5:36 p.m., H.S. 75 in 75. As a result of the centrifugal treatment the Stentors from B had their granules shifted markedly to one side and showed two zones plainly, those from A showed no movement of granules.

December 10. At 7:51–7:54 p.m. Stentors were placed into A and B. A contained 6 cc. $\frac{M}{32}$ NH_4Cl, B contained 6 cc. $\frac{M}{40}$ $CaCl_2$. The Stentors from A and B were centrifuged at 8:24 p.m., H.S. 75 in 75. Those from A showed little or no movement of granules, those from B had their granules shifted to one side of the cell.

An experiment in which potassium chloride was compared with calcium chloride was indecisive, for the Stentors in the potassium chloride solution were with one exception broken up on centrifugal treatment. The action of potassium chloride was compared with that of sodium chloride by using each of these salts in combination with calcium chloride. The calcium chloride tends to prevent the coagulating action of the sodium or potassium salts, but in the case of the latter salt it does not exert quite as marked a protective action. This indicates that potassium chloride is a somewhat stronger coagulant than sodium chloride.

January 4, 1922. Four dishes were used, A, A′, B, B′. Each of these dishes contained 2 cc. $\frac{M}{40}$ $CaCl_2$. In addition 3 cc. $\frac{M}{32}$ NaCl were added to A and 3 cc. $\frac{M}{32}$ KCl to A′, whereas 4 cc. $\frac{M}{32}$ NaCl were added to B and 4 cc. $\frac{M}{32}$ KCl to B′. Stentors were first washed in distilled water (conductivity water) and then placed into A, A′, B, B′, at 3:47–3:48 p.m. At 4:15 p.m. Stentors from B and B′ were centrifuged simultaneously, H.S. 80 in 79. The Stentors from B were found to have their granules well shifted to one side whereas those from B′ showed no trace of granular movement. At 5:03 p.m., Stentors from A and A′ were centrifuged simultaneously, H.S. 75 in 75. As a result of this treatment, Stentors from A showed a marked movement of granules to one side with the resultant formation of two zones; Stentors from A′ showed little if any movement of granules.

From the above experiment it may be concluded that potassium chloride is a more vigorous coagulant than sodium chloride. Both salts are antagonized by calcium chloride. Apparently only a small amount of calcium chloride is necessary to counteract a much larger quantity of sodium chloride. Inasmuch as the Stentors were grown in tap water rich in calcium salts, care should have been taken in every case to remove all traces of tap water when the Stentors were trans-

ferred from their culture solution into any solution whose action was being tested. Such a precaution was not taken in the November and December experiments reported above. If Stentors are first washed in conductivity water and are then placed in solutions of sodium or lithium chloride the coagulative action of these salts is more rapid and pronounced than when some tap water is carried over with animals. Under such conditions, however, centrifuge tests are difficult to perform, for the Stentors tend to break into fragments.

The experiments so far have shown that calcium and magnesium chlorides do not exert the coagulating action manifested by sodium, potassium, lithium, and ammonium chloride. It has also been pointed out that potassium chloride is a more vigorous coagulant than sodium chloride. In order to determine the relative position of calcium and magnesium chlorides, the action of these salts in preventing coagulation was compared. It was found that calcium chloride was more effective than magnesium chloride in this respect.

January 11, 1922. Four dishes were used A, A′, B, B′. Of these A contained 0.2 cc. $\frac{M}{40}$ $CaCl_2$ + 6 cc. $\frac{M}{32}$ KCl, A′ contained 0.2 cc. $\frac{M}{40}$ $MgCl_2$ + 6 cc. $\frac{M}{32}$ KCl, B contained 0.2 cc. $\frac{M}{40}$ $CaCl_2$ + 6 cc. $\frac{M}{32}$ NH_4Cl, B′ contained 0.2 cc. $\frac{M}{40}$ $MgCl_2$ + 6 cc. $\frac{M}{32}$ NH_4Cl. Stentors were first washed in conductivity water and then placed into A, A′, B, B′ at 3:25–3:26 p.m. At 4:20 p.m. Stentors from A and A′ were centrifuged simultaneously, H.S. 75 in 75. Those from A showed a slight stratification as a result of granule shifting. Those from A′ showed no stratification, most of them showed injuries at various parts of their periphery. At 4:36 p.m. Stentors from B and B′ were centrifuged simultaneously, H.S. 75 in 75. The Stentors from B showed no stratification at all, those from B′ were all disintegrated.

This experiment shows that calcium is a more powerful anticoagulant than magnesium. It also indicates that ammonium probably exerts a more vigorous coagulating action than potassium. This follows from a comparison of the centrifuge tests made on Stentors from A and B. These tests were of the same duration although some time elapsed between the two tests.

Summing up the evidence, it appears that when the chlorides of various metals are compared, their power of coagulating Stentor protoplasm varies in accordance with the following series:

$$Ca < Mg < Na < K < NH_4$$

This series is very nearly the same as the one previously obtained for sea-urchin eggs. The only difference lies in the fact that in this series sodium and potassium have changed places.

When these series are compared with the series of cations arranged in the order of their adsorption by egg albumen (see p. 484), it is seen that the order is the opposite in the two cases. The series of cations arranged in the order of their adsorption by egg albumen is

$$Ca > Mg > K, Na > NH_4$$

This series was obtained by comparing the precipitating power of the various cations in alkaline medium, under which conditions the protein is presumably changed with the negative sign.

For protoplasm the order of precipitating power is exactly the reverse for as has been shown

$$Ca < Mg < K, Na < NH_4$$

According to colloid chemical theory, this indicates that the protoplasmic colloids are positively charged. Of the various cations used, calcium and magnesium are doubtless adsorbed most readily. When living cells are placed in contact with calcium or magnesium chloride, there is a greater adsorption of cations than when such cells are placed in contact with sodium or ammonium chloride. Calcium and magnesium would therefore tend to increase the positive charge of the colloidal particles. Such an increase in positive charge would tend to coagulate protoplasm if its original charge were negative. The fact that calcium and magnesium ions prevent rather than aid coagulation shows that normally the colloidal particles of protoplasm bear a positive charge.

In the discussion so far, only the interior of protoplasm has been considered. The outer surface layer is different from the interior (see p. 483). Sea-urchin eggs are surrounded by a thin outer membrane which can best be regarded as a precipitation membrane.[18] This membrane, the vitelline membrane, is charged not positively, but negatively. The very salts which favor coagulation of the egg cell interior cause the opposite sort of changes in the vitelline membrane. Thus sodium chloride causes the membrane to absorb water and swell,[19] and ammonium chloride has the same effect. Calcium chloride does not have any such swelling action and it tends to inhibit the swelling produced by sodium chloride.[20]

[18] Heilbrunn, 1915, Biol. Bull., xxix, 149.

[19] Heilbrunn, c.

[20] R. S. Lillie, 1911, This Journal, xxvii, 289; 1911, Journ. Morph., xxii, 695. In these papers Lillie failed to recognize the effect of sodium chloride as a swel-

These observations indicate that the vitelline membrane of the sea-urchin egg cell is negatively charged. In fact, we may think of a typical cell as being composed of an interior protoplasmic mass composed of colloids bearing positive charges, and an external layer bearing negative charges. This would be in accord with the view expressed in 1915 that the outer layer of the cell is a precipitation membrane.

In Stentor also it is probable that the outer layer of the cell is negatively charged. Sodium, potassium and ammonium chloride each causes the cilia to swell and probably causes swelling of the underlying membrane also. This swelling action is prevented and antagonized by calcium chloride or magnesium chloride.[21]

It is probable that in some solutions the negative charge of the surface layer may be changed by adsorption of cations to a positive charge. Such a phenomenon apparently may occur in blood cells when these are subjected to the influence of salts with trivalent cations. In sea-urchin eggs also there is some evidence that strongly adsorbed cations may alter the charge at the surface. Thus under some conditions solutions of pure calcium chloride appear to have this effect.

From our experiments we have concluded that the isolated living cell is composed of an interior mass consisting of colloids charged positively, bounded by an external layer whose colloids are negatively charged. There is a considerable mass of biological information which can be brought forward to support this conclusion.

As pointed out previously (see p. 484), Barratt failed to settle the question as to whether protoplasm is acid or alkaline, for his indicators could not be relied upon in the presence of such concentrated colloidal solutions as are found in living substance. Indicator methods may however be used to determine the hydrogen ion concentration of the cell sap in the interior of large and watery plant cells. This cell sap is really not protoplasm itself, for it is a vacuole, but it is not unlikely that it has a similar hydrogen ion concentration. The measurements of Rohde[22] and Collander[23] show that in most cases the cell sap is decided acid although sometimes it may be nearly or even quite neutral. Of course, excess of hydrogen ions would favor a positive charge.

ling. He confused membrane swelling and membrane elevation, and the two processes were first distinguished by Heilbrunn, 1915, l.c. Lillie has not followed this distinction, see Lillie and Baskerville, 1921, This Journal, lvii, 110.

[21] Similar effects have been described for the cilia of marine forms by R. S. Lillie, 1904, This Journal, x, 419, and 1906, This Journal, xvii, 89.

[22] K. Rohde, 1917, Arch. f. d. gesammt. Physiol., clxviii, 411.

[23] R. Collander, 1921, Jahrb. f. wiss. Bot., lx, 354.

More direct evidence has been furnished recently by Kahho.[24] He found that in their power to kill plant protoplasm the cations are effective in the following series:

$$K > NH_4 > Na > Sr, Mg, Ba, Ca.$$

Thus Kahho's work agrees with mine in showing that the monovalent cations are more toxic than the bivalent. Kahho assumes that death is due to coagulation and this assumption is probably correct. In his interpretation of his experiments, Kahho considered as a possible explanation the view that protoplasm might be acid or positively charged. He rejected this conception in favor of an explanation on the basis of differential permeability. Such an explanation can not account for the results obtained in the experiments on sea-urchin eggs described above (for a discussion of this point see page 486). Therefore Kahho's explanation is probably not the correct one and his results can best be explained by assuming that protoplasm is positively charged.

Additional support for this view is to be found in the ordinary knowledge concerning vital stains. It is a well-known fact that the dyes best suited for staining living protoplasm all have an alkaline reaction. It is true that there are some acid dyes which color the living cell, but all of the dyes which experience has shown best for vital staining are alkaline. Thus neutral red, methylene blue, Janus green, have an alkaline reaction. According to von Möllendorff[25] who has recently reviewed the entire subject of vital staining, the acid vital stains do not really color the constitutents of the protoplasm at all. According to our view, it is easy to understand why the alkaline dyes, which are generally charged negatively, should be particularly effective as vital stains. Colloids with opposite electric charges precipitate each other. When the negatively charged alkaline dyes enter the cell they immediately form precipitation compounds with the positively charged colloids of protoplasm. Due to this precipitation, alkaline dyes are stored up very readily by the living cell.

It might be thought that the passage of an electric current through protoplasm would furnish information as to the electric charges of protoplasmic colloids. Hardy[26] found that when an electric current was passed through an onion root tip, the cytoplasm usually collected

[24] H. Kahho, 1921, Biochem. Zeitschr., cxx, 125.
[25] W. v. Möllendorf, 1920, Ergebn. d. Physiol., xviii, 141.
[26] W. B. Hardy, 1913, Journ. Physiol., xlvii, 108.

at the end of the cell toward the cathode. On the other hand Meier[27] claims that in the root tips of pea, onion and bean, an electric current usually causes a migration of cell contents toward the anode. In interpreting experiments of this sort it must be remembered that the interior and exterior of protoplasm probably differ in their electric charge. Studies of the migration of isolated cells in an electric field have in general shown a migration toward the anode.[28] This is to be expected on the basis of our view that the surface of the cell is charged negatively. When cells are suspended in a solution of moderately good conductivity and an electric current is passed through the solution certainly most of the current follows the course of least resistance and passes around the cells; only a small fraction of it passes through them. Hence the charge on the surface layer very largely determines the direction of migration, and this is the reason that in almost every case cells migrate toward the anode. We would expect however that in larger cells, in which the surface area is of course relatively small compared to the interior, the charges of the interior protoplasm might become significant. It is interesting to note that Lillie[29] found that when frog blood cells were immersed in sugar solution the later, but not the smaller, leucocytes migrated toward the cathode. This fact is in perfect accord with the views expressed above.[30] If the interior of protoplasm were positively charged, we would expect that the passage of an electric current through a very large cell would tend to make it bulge out at the cathode. This is exactly what has been found to occur in Amoeba and Paramecium, when strong currents are passed through these animals.[31]

Whether one accepts our interpretation of the electric charges of protoplasm or not, the experimental data of this paper provide a clear explanation of the facts of antagonism. Every physiologist is aware of the fact that sodium chloride and calcium chloride in very many cases

[27] H. F. A. Meier, 1921, Bot. Gaz., lxxii, 113.

[28] R. S. Lillie, 1903, This Journal, viii, 273; R. Höber, 1904, Arch. f. d. gesammt. Physiol., ci, 607. For references to more recent literature see E. Putter, 1921, Zeitschr. f. Immunitätsforsch., xxxii, 538.

[29] R. S. Lillie, l.c.

[30] Lillie's own explanation was different. He believed that the nucleus bore charges opposite to sign to those of the cytoplasm, and that the nucleus was relatively large in the small cells. But such an interpretation would not explain the migration of human red blood cells to the anode (Höber, l.c.).

[31] H. S. Jennings, *Behavior of the Lower Organisms*, New York, 1906; see p. 12 and p. 84.

produce opposite effects upon protoplasm. In general, monovalent cations are "antagonized" by bivalent cations. The literature on the subject of this antagonism is very large. Some of it has been summarized by Höber.[32] All sorts of explanations have been urged to explain this widespread phenomenon. Even radio-activity has been considered as a possible explanation. Generally speaking, the theories of antagonism have been based on sheer speculation. Höber has doubtless led the way toward a rational colloid chemical explanation,[33] but he had no facts at his disposal concerning the effects of various ions on the protoplasm in the interior of cells. He assumed that sodium liquefies and calcium coagulates. The results of this paper provide a clear-cut explanation of the phenomenon. Sodium, potassium and ammonium coagulate the interior of protoplasm, calcium and magnesium tend to liquefy it. This is shown by actual viscosity tests.

SUMMARY

1. By a standard method of colloid chemistry it is shown that the colloids in the interior of protoplasm bear a positive electric charge.

2. The exterior layer of protoplasm is charged negatively.

3. These conclusions are supported by, and themselves help to interpret, many observations previously recorded in the literature.

4. The antagonistic action of salts on protoplasm is explained by actual data concerning the effect of these salts on protoplasmic viscosity.

[32] R. Höber, Physikalische Chemie der Zelle und der Gewebe, 4te Auflage, Leipzig 1914.

[33] R. Höber, l.c.; also 1920, Arch. f. d. gesammt. Physiol., cvxxxii, 104.

THE INFLUENCE OF PARATHYROIDECTOMY ON GASTRIC SECRETION

SILBER C. PEACOCK AND LESTER R. DRAGSTEDT

From the Hull Physiological Laboratories of the University of Chicago

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The complete removal of the parathyroid glands in dogs, in most cases, causes the development of a series of symptoms, usually referred to as parathyroid tetany because of the striking character of the nervous manifestations, but in which there is also a group of symptoms indicating disturbances in the gastro-intestinal tract. Especially noticeable is the profuse salivation, anorexia, vomiting and diarrhea. Clinical tetany is often associated with diverse gastro-intestinal disorders, particularly in children, and there is considerable evidence indicating a primary etiologic relation of the intestinal disorder and the tetany. Carlson (1) found that, contrary to the statements of Falta and Kahn in regard to certain cases of human tetany, there is depression in the motor activity of the stomach and intestines in parathyroid tetany in cats and dogs, and in severe tetany there may be complete paralysis or atonicity. He also found a secretory depression which in severe cases results in a practical failure of digestion. Keeton (2) confirmed these findings in a study of the gastric secretion of parathyroidectomized cats provided with a Pawlow accessory stomach. He found a depression in the quantity of gastric juice, the free and total acidity, and the pepsin concentration, roughly in proportion to the severity of the tetany.

The control and cure of parathyroid tetany in dogs by the dietary regimen introduced by Dragstedt (3) and by the repeated intravenous injection of Ringer's solution discovered by Luckhardt (4) make it certain that animals in parathyroid tetany are suffering from an intoxication. Furthermore the experiments of Dragstedt and Peacock (5) show that the chief source of the intoxication is the alimentary tract. While it is possible that there is some direct relation between the functions of the parathyroid glands and the gastro-intestinal tract, the experimental evidence suggests that the depression

in the motor and secretory functions of the stomach and intestines is produced by the depressant action of the tetany toxins in the blood rather than by the loss of a possible regulatory hormone from the parathyroid glands. The prevention of tetany in parathyroidectomized dogs by dietary control affords a method of studying gastric function in dogs, deprived of a possible parathyroid hormone, but not suffering from a profound intoxication.

EXPERIMENTAL PROCEDURE. Pawlow accessory stomach pouches were prepared in four strong adult female dogs. As soon as possible the animals were placed on a diet consisting of 500 cc. of skim milk, 500 cc. of lactose solution (23 per cent), and white bread, ad libitum. The diet was taken voluntarily for the most part, but occasionally it was necessary to give the milk and lactose by stomach tube. The feces soon became liquid in character, odorless or possessed a faint fruity odor, acid to litmus, with the fecal bacteria of the aciduric type both in morphology and staining reactions. The diet was continued for 10 to 12 days, and the daily secretion from the pouch collected for the 2-hour period following each feeding. After the normal response had been thus determined, the thyroid and parathyroid glands were removed, and the subsequent effect on the gastric secretion noted. The diet was continued as before and for the most part the animals showed normal appetite and desire for food. In no case did the thyroparathyroidectomy have any appreciable depressant effect on the gastric secretion. A detailed record of one typical experiment is given in the following table. 1.

DISCUSSION. The experiments demonstrate that the complete removal of the thyroid and parathyroid glands does not diminish the gastric secretion, in amount or in acid concentration, when the usual post-operative tetany is controlled by diet. There is thus no evidence of a parathyroid hormone necessary for normal gastric secretion. The depression that occurs when tetany develops is undoubtedly a secondary effect of the tetany poisons in the blood. It is probable that these depress the local gastric secretory mechanism directly. It is also evident that the depression in the motor activities of the stomach and intestines described by Carlson in parathyroid tetany is a secondary effect of the intoxication, rather than a primary factor, since the parathyroidectomized dogs showed no motor abnormalities, when the tetany was not permitted to develop. They continued in good nutrition and rapidly gained in weight, a fact which indicates that intestinal digestion and absorption was entirely normal.

TABLE 1

Gastric secretion following thyro-parathyroidectomy

Diet: Skim milk 500 cc., 23 per cent lactose solution 300 cc., white bread ad libitum.

Gastric juice: Total from pouch for 2-hour period following feeding.

DAYS	GASTRIC JUICE			REMARKS
	Amount	Free acid	Total acid	
	cc.			
1	18	0.2006	0.2918	
2	14	0.2812	0.3724	
3	12	0.3646	0.4649	
4	15	0.3281	0.3889	
5	10	0.3007	0.3737	
6	15	0.4102	0.4649	
7	13	0.3281	0.3792	
8	11	0.3463	0.3828	
9	10	0.3281	0.3646	
10				Thyro-parathyroidectomy
1	9	0.2827	0.3921	
2	13.5	0.4284	0.4740	Slight tetany, recovery
3	14	0.4512	0.5059	Tremors, no tetany
4	9	0.4192	0.4785	Severe tetany. 325 cc. Ringer's solution given intravenously
5	4	0.3281	0.4010	No tetany
6	14	0.4558	0.5561	No tetany
7	6	0.3326	0.4113	Tremors
8	10	0.3646	0.4375	Spasticity. 400 cc. Ringer's solution given intravenously
9	25	0.4071	0.4928	No tremors, no spasticity
10	42	0.3919	0.4374	Animal apparently normal
11	31	0.3549	0.4238	Animal apparently normal
12	16	0.3990	0.4557	Animal apparently normal
13	30	0.4193	0.4539	Animal apparently normal
14	14	0.4010	0.4420	Animal apparently normal
15	20	0.3007	0.3828	Animal apparently normal
16	45	0.3916	0.4373	Animal apparently normal
17	28	0.4010	0.4602	Animal apparently normal
18	48	0.3463	0.4101	Animal apparently normal
19	30	0.3649	0.4192	Animal apparently normal
20	17	0.3919	0.4611	Animal apparently normal
21	18	0.2553	0.3190	Depressed. Severe enteritis
22	31	0.3828	0.4193	No improvement
23	20	0.3919	0.4375	Depressed. Bloody diarrhea
24	2	0.3463	0.3828	No improvement
25				Death

CONCLUSIONS

Complete extirpation of the thyroid and parathyroid glands in dogs has no depressant effect on gastric secretion if the development of tetany or depression be prevented by dietary control.

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EXTIRPATION OF ADRENAL GLANDS IN ALBINO RATS

JOHN T. LEWIS

From the Institute of Physiology, Faculty of Medicine, Buenos Aires

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In most species total extirpation of the adrenal glands is followed by death within a few hours. Adrenal insufficiency has been proved to be the cause, but in what this insufficiency consists is extremely difficult to ascertain. Up to now it has been impossible to state exactly what is the primary cause of death after adrenal extirpation.

Chronic experimental adrenal insufficiency permits study of the question under different conditions and may lead us to a satisfactory explanation.

In a few species a certain number of individuals survive total adrenal extirpation. In these individuals there is apparently no adrenal insufficiency, the functions of these glands are maintained by accessory suprarenals or by a different, but unknown process. Close observation of the period immediately following adrenal extirpation may permit us to discover some functional alteration not at once compensated, and so give us a clue as to the nature of the intimate mechanism of adrenal insufficiency and the functions of these glands.

We have undertaken this study in albino rats. Phillipeaux (1) and Harley (2) observed that double adrenal extirpation does not invariably occasion death in this species. Boinet (3) confirmed this fact; 50 per cent of a numerous series lived several months after suppressing both suprarenals.

A. and H. Cristiani (4) and Strehl and Weiss (5) find evidence contradictory to these statements: extirpation of both adrenals is followed by death within a few hours.

EXPERIMENTS. In more than 400 albino rats both adrenals were extirpated. We used a simple technic: Under ether anesthesia a longitudinal dorsal incision of about one inch is made, the middle being situated on a level with the last rib. In the angle formed by this rib with the spinal column, the muscles are separated by introducing curved forceps shut, and then opening them. With a little practice the supra-

renal will be found immediately below; at first it is convenient to look for the kidney, above which the adrenal is situated. The adrenal is then separated from the surrounding tissues and removed. No ligatures are used; a correct operation provokes not the slightest hemorrhage. It is not necessary to sew the muscle; a stitch in the skin is sufficient. Both adrenals were always extirpated in one session.

Certain details must be observed: First, animals must be healthy; epidemic bronchopneumonia is frequent in rats and must be carefully avoided.

Second, animals recently operated are extremely sensitive to low temperature. Working in winter it is necessary to keep them in a warm room.

Third, food must be specially looked after. Bread and milk twice a day in abundant quantities was found to be an excellent diet.

We obtained the following results: Mortality has varied from 20 per cent to 40 per cent. Death generally occurring in the first 48 hours, exceptionally 3 or 4 days later. Age and sex have apparently no influence. During a few days after operation the animals are sluggish and huddle up together, but soon these symptoms disappear and they are quite normal.

Pregnancy and parturition have been repeatedly observed, offspring being perfectly normal. In this respect we can confirm Cristofoletti's conclusions.

Growth is not hindered. Half the individuals of several families were operated on a short time after birth (30 to 40 days), their weight increased regularly and parallel to that of controls, some females became pregnant from adrenalectomized males and had normal offspring.

Schwarz (6) maintains that in adrenalectomized rats a marked diminution of the glycogenetic power of the liver is observed. According to Kuriyama (7) this is not exact. The glycogenetic power of the liver is not impaired by adrenalectomy. Catan, Houssay and Mazzocco (8) have found a diminished quantity of glycogen in both liver and muscles, and a slight hypoglycemia during the first 10 days following operation in our rats. This alteration can not be explained by anesthesia or trauma as it is not observed in animals with only one adrenal removed. It is however a symptom that disappears quickly; in a month or less time normal quantities of sugar are found.

From these facts it would seem that double adrenalectomy in albino rats provokes scarcely any symptoms, and that adrenal insufficiency, if it exists, is rapidly compensated, perhaps by the numerous accessory

bodies possessed by this species (Wiesel) or by some other, but up to now unknown, mechanism. In the next paper we shall see that this normal state is only apparent. If a more careful study into the function of these animals is made at least one abnormal symptom of certain duration may be detected.

CONCLUSIONS

1. Albino rats survive double adrenalectomy in a proportion varying from 60 per cent to 80 per cent.
2. Sluggishness and a particular sensibility to cold are observed in the first few days following operation.
3. Growth is not hindered, even when the adrenals are removed at an early age (30 to 40 days).
4. Reproductive functions, fertilization, pregnancy and parturition are apparently normal.
5. After a slight alteration that passes in a few days, liver glycogen and glycogenetic power are normal.

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SENSIBILITY TO INTOXICATION IN ALBINO RATS AFTER DOUBLE ADRENALECTOMY

JOHN T. LEWIS

From the Institute of Physiology, Faculty of Medicine, Buenos Aires

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Much has been written about an antitoxic function of the adrenal bodies. The belief that this function exists is founded on the following facts: First, alterations observed in the gland tissue after absorption of poisons; second, neutralization of poisons by adrenal extracts and by adrenalin; third, higher sensitiveness to poisons after adrenalectomy.

1. Alterations observed in adrenals after absorption of poisons. Roux and Yersin (1) observed the existence of intense congestion in the adrenal bodies of guinea pigs killed by injection of diphtheria toxin.

Bernard and Bigart (2) have studied in detail alterations produced in these glands by poisoning rabbits with arsenic, mercury, copper, zinc, iodine and lead, using large doses. Hemorrhage, necrosis, disappearance of pigment and lipoid are observed. With small doses, especially if repeated, hypertrophy is found: numerous cells are dividing, many ergastoplasmic figures are to be found, pigment is abundant and the cortical cells have a typical spongiocytic aspect. In the first case there is a state of "hypoepinephria"; in the second, of "hyperepinephria." These changes take place in the cortex, the medulla remaining unaltered except for some slight vascular reactions.

Charrin (3) and Oppenheim and Loeper (4) using bacterial cultures and toxins, have obtained similar results.

2. Neutralization of poisons by adrenal extracts and by adrenalin. Langlois and Charrin (5) and Myers (6) have found that mixing "in vitro" nicotine or cobra venom with suprarenal extract a higher dose of the poisons is necessary to kill animals. Boinet (7) does not confirm this statement.

Marie (8) has studied in detail neutralization of tetanic and diphtheria toxin by adrenalin. Burke, Elder and Pischel (9) have used botulin poison with similar results.

Recently Tawara (10) has proved that hydrochloric acid contained in adrenalin solutions is the active principle in these experiments.

By injecting extracts or adrenalin previously or with the toxic substance, Oppenheim (11) and Falta and Lucovic (12) obtain a certain protection against poisoning. Camus and Porak (13) fail to confirm these results.

3. Higher sensibility to poisons after adrenalectomy. Albanese (14) found that the minimal fatal dose of neurin was ten times smaller for frogs whose adrenals had been recently destroyed, than for normal animals. No difference was found for strychnin and atropin. Abelous (15) finds higher sensitiveness to atropin in adrenalectomized frogs a very short time after the operation. Boinet (16) confirms in rats Albanese's statement on neurin and extends it to ouabaio poison. Schwarz (17) in this same species finds that adrenalin is more toxic after extirpation of both adrenals.

Camus and Porak (18) used rabbits and observed higher sensitiveness to strychnine and curare a few hours after double adrenalectomy.

Marie and Morax (19) provoked in a guinea pig symptoms of tetany, shortly after double adrenalectomy with a dose of tetanus toxin harmless for controls.

Extirpation of one adrenal according to Charrin and Langlois (20) and Oppenheim (21) not only does not increase the sensitiveness of guinea pigs to divers bacterial toxins but increases their resistance.

EXPERIMENTS. We have used healthy adult albino rats whose adrenals were removed according to the technic described in a previous paper. The minimal fatal dose for subcutaneous injection of various poisons was established, expressing it in milligrams per gram of animal subtance. Several animals were used to avoid errors due to individual sensitiveness. The first few days were allowed to pass in order to exclude the influence of anesthesia, trauma, etc. In table 1 are detailed the results obtained.

Splenectomy, extirpation of one kidney, surgical trauma of the adrenal region without adrenal extirpation, do not alter the sensitiveness to poisons (19 experiments).

Removal of only one adrenal, right or left, does not affect poisoning by cobra venom. Some animals under these conditions die with weaker doses of morphin than the controls, but generally no difference is observed.

To establish for how long after double adrenalectomy this alteration persists, different doses of morphin were injected in rats operated on a long time before, with the results shown in table 2.

Action of extracts: Cobra venom was mixed with a 10 per cent watery extract of guinea pig adrenal and left in contact from 40 minutes to 2 hours at room temperature. Sometimes extracts were filtered through cotton-wool before, others after contact; on other occasions the mixture was injected without filtering. In no instance was there a diminished activity of the venom.

TABLE 1

TOXIC SUBSTANCE	DELAY AFTER OPERATION	MINIMAL FATAL DOSE FOR: mg. per kg.		DIFFERENCE	NUMBER OF RATS USED	NUMBER OF CONTROLS
		Operated rat	Control			
	days					
Cobra venom	5–7–10	0.0001	0.0005	5 times more	24	27
Curare	8	0.007	0.014	2 times more	10	16
Veratrin	8–10	0.002	0.015	7 times more	8	13
Morphin	8, 10, 13, 15, 30	0.001	0.4–0.5	400–500 times more	28	17
Digitoxin	15	0.00025	0.001	4 times more	5	9
Diphtheria toxin*	15	200 MLD for guinea-pig	Not sensitive	—	3	2
Adrenalin	8	0.0001	0.0005	5 times more	4	4
Strychnine	7–9–11	0.002	0.003	—	12	12
Picrotoxin	15	0.005	0.005	—	6	6

* Controls were made to assure that the phenol used to preserve the toxin was not the active substance.

Papaverin and codein were found to be at least thrice more active in operated animals than in controls, but the minimal fatal dose was not exactly established.

TABLE 2

TIME AFTER OPERATION	PER GRAM	
45 days	0.1	Survival
60 days	0.3	+
6 months	0.3	Survival
6 months	0.4	+
7 months	0.1	+

Injection of 10 per cent watery extract 20 minutes before a minimal fatal dose of cobra venom offers no protection.

Discussion. Previous evidence of an increased sensitiveness to poisons after adrenalectomy is not conclusive for two reasons: *1*, with the exception of Boinet's experiments, all were conducted under acute conditions and therefore the influence of anesthesia, trauma, etc., could not be removed; *2*, a very small number of experiments, sometimes only one (Marie and Morax, Abelous), was reported, so that the results might have been due to secondary causes not suspected.

From our experiments we can conclude that adrenalectomy produces in albino rats an increased sensitiveness to several poisons (morphin, curare, cobra venom, digitoxin, papaverin, codein, diphtheria toxin, veratrin, adrenalin) sometimes as with morphin from 400 to 500 times the normal sensitiveness. With other poisons (strychnine, picrotoxin) this is not observed.

Splenectomy, extirpation of one adrenal and surgical trauma of the adrenal region have no such influence. Olds (22) has found that thyroidectomy does not diminish resistance to morphin in rats. Therefore we may assert that adrenalectomy is the specific cause of this abnormal state. The great number of our experiments excluded all secondary factors that may have passed unknown.

As time passes after operation, this increased sensitiveness diminishes and ultimately disappears. Thus are explained Stewart and Rogoff's (23) objections to our statements. They have evidently not read our second paper on this subject (see Compt. Rend. Soc. Biol., 1921, lxxxv, 685).

Giusti (24), having taken the same care as ourselves to avoid all errors, has established similar facts in toads (Bufo marinus).

We have given some evidence (25) that adrenalectomized dogs are more sensitive to morphin. We may therefore affirm that an increased sensitiveness to intoxication is a characteristic symptom of adrenal insufficiency.

It has not been found that extirpation of one adrenal increases resistance to poisons, as Oppenheim (26) and Charrin and Langlois (27) maintain. Generally no alteration follows, sometimes a greater sensitiveness to morphin is observed, but not constantly.

Protection against poisons is not obtained by previously injecting adrenal extract or by mixing it with the toxic substances before injecting. Even if neutralization were constantly observed it could not be considered as a proof that the gland has an antitoxic function. Diez and Lerda (28) have established that tetanus toxin is neutralized by a great number of tissues and places them in the following order according to their activity: rectal mucosa, kidney, spleen, stomach, intestines, spinal cord, liver, adrenal gland, muscle, lung, blood serum. Nobody will infer from this that an important antitoxic function is accomplished in the rectum. It is simply due to a physical or chemical property of the tissues. Much care must be taken in drawing conclusions on the functions of organs from experiments carried out with extracts of their tissues or pharmacological preparations. Exner (29)

remarks that injection of adrenalin or suprarenal tissue extract produces local vasoconstriction and so delays absorption; in this way may be explained some experiments that pretend to prove an antagonism between poisons and adrenalin.

Two facts serve to demonstrate an antitoxic function of the adrenals: hypertrophy of the gland cortex during chronic intoxication and increased sensitiveness to poisons after adrenalectomy.

This last fact could be explained in another way than by admitting that it is due to defective neutralization of poisons. It may be that adrenalectomy so alters the physiological equilibrium that destruction or elimination of toxic substances is hindered. But it seems to us more probable that the cells are more sensitive owing perhaps to an altered permeability. Further research is being carried on in this direction.

SUMMARY

1. Double adrenalectomy in albino rats increases their sensitiveness to morphin, codein, papaverin, curare, cobra venom, veratrin, digitoxin, adrenalin, diphtheria toxin. With picrotoxin and strychnine no difference is observed.

2. This abnormal state appears immediately after operation. After a certain lapse of time it gradually diminishes until complete disappearance.

3. Extirpation of one adrenal occasionally increases sensitiveness to morphin, generally it has no effect on resistance to poisons.

4. Mixture "in vitro" and previous injection of watery extract of suprarenals have not given protection against poisoning.

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THE RELATIVE IMPORTANCE TO LIFE OF CORTEX AND MEDULLA OF THE ADRENAL GLANDS

B. A. HOUSSAY AND J. T. LEWIS

From the Institute of Physiology, Faculty of Medicine, Buenos Aires

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Loss of both adrenals provokes death in a few hours in nearly all species. In dogs within 1 to 3 days (average, 26 hours) according to our own experiments. In man alterations of these glands occasion death in a short time (Sergent-Bernard's syndrome) or else bring about Addison's disease, of longer duration but not less fatal. The suprarenals must therefore be considered as organs essential to life. But as they are formed by two different tissues, cortical and medullary, the question arises as to which of them plays a principal part.

Some authors maintain that the medulla is the vital tissue, others that it is the cortex. Pende believes that both are necessary to life. We will examine the evidence brought to support these different opinions and afterwards discuss them with the help of our own experiments on this subject.

Predominance of medulla. This tissue secretes a substance of powerful pharmacological activity, adrenalin; it is therefore natural to presume that it is of great importance, especially if we consider that the functions of the cortex have not yet been clearly established.

A. and H. Cristiani (1) observed that extirpation of both adrenals provokes death in rats, and that these animals survive if extirpation is incomplete, provided that in the part left behind, medullary tissue is found in good condition. They also observed that suprarenal grafts are unable to maintain life in adrenalectomized rats, although only the medullary tissue degenerates, the cortex being found in excellent condition. This evidence led them to believe that the medulla is essential to life.

Vassale and Zanfrognini (2) removed all the medullary tissue leaving only the cortex in cats and rabbits and found that this operation is as fatal as double adrenalectomy. Vassale (3) claims that extirpation of one adrenal and the abdominal paraganglia is always followed by death.

Levi della Vida (4) has endeavored to destroy both tissues separately by means of cytotoxic sera. When a serum that has been prepared by injecting medulla into dogs or ducks, is administered to a guinea pig, the animal dies, great alterations being found in both adrenals, but only in the medulla, the cortex remaining almost intact. Serum prepared with cortex is almost harmless; it brings about only slight alterations.

Predominance of cortex. Biedl (5) has brought out important evidence on this point. In dogs incomplete adrenal extirpation permits survival when an eighth of the adrenal tissue is left behind, provided that this portion consists of cortex. If all the cortex is removed, leaving the medulla as intact as possible, death occurs as after total adrenalectomy.

In Elasmobranchs both tissues are found separated. The cortex forms Balfour's inter-renal body and the medullary tissue is disseminated in numerous small bodies. Extirpation of the inter-renal body provokes death, although all the chromaphil tissue remains.

Swale Vincent (6) and Pettit (7) repeated these experiments in eels, obtaining different results. Later Giacomini (8) described a cranial portion of the cortical tissue in these animals and so explained these last authors' results; they had made only incomplete extirpation of the inter-renal tissue, therefore their animals survived.

Pende (9) has proved that denervation of the adrenals is followed by atrophy of the medulla. Stewart and Rogoff (10) removed one adrenal and cut all nerves going to the other in cats, dogs, rabbits and monkeys. Five weeks later no adrenalin could be found in the blood taken from the capsular vein although their test method was sensitive up to 1:1000 millions. These animals had no abnormal symptoms, so that we can deduce that adrenalin is not indispensable to life and to the normal functioning of the organism.

Wheeler and Vincent (11) completely removed the left adrenal, and amputated half the right gland, cauterizing its medulla. Three weeks or a month later the remaining gland tissue was extirpated, stained with Müller's fluid and examined for the presence of medullary tissue. A total of 32 dogs, 24 cats and 4 rabbits were operated. In all cases where death was due to operation and not to accident, such as loss of blood, anesthesia, etc., extensive damage to the cortex had been done. In several instances, where no symptoms had been observed, all or practically all the medulla had been removed.

Necessity of both portions. Pende (12) believes that the compound organ is necessary to life. He has observed that grafts maintain life in cats after adrenalectomy for a longer time when both tissues live in the graft. These experiments are not convincing because it is not possible to arrive at a definite conclusion whether the association of cortex and medulla is or is not always more efficient in grafts than the presence of one only of the tissues. Pende supposes the existence of certain functions realized by this association, but up to the present nobody has been able to establish in what they consist.

Experiments. We have used dogs in our experiments for several reasons: *1*, Total adrenalectomy always provokes death in a short lapse of time (from 1 to 3 days, 26 hours mean); *2*, it is possible to remove all the medulla leaving the cortex behind; *3*, their size makes the operation easy and permits divers physiological observations.

Following our technic it is possible to obtain survival in the great majority of cases, after total extirpation of the adrenal medulla.

First the left adrenal medulla is removed. For this the left suprarenal is exposed through a dorsal incision, the vein is separated and both ends of the gland are dissected, cutting the splanchnic nerve, but leaving a good vascular pedicle on the internal side. This pedicle is taken up with a curved clamp, so as to prevent bleeding and to hold the gland during the rest of the operation. The gland is then opened lengthwise, with a sharp Gillette blade, as if it were a book. Medulla and cortex can be easily differentiated, and all the medulla removed with a spoon. It is convenient to remove also a part of the cortex so as not to leave any chromophil tissue behind. The gland is sewed up with catgut and the body wall closed.

A week or ten days later the right adrenal is removed.

In a first series 16 dogs were operated in this way. Three (nos. 4, 7 and 9) died within 48 hours, with symptoms of total adrenalectomy. Severe damage had been done to the cortex. One (no. 1) ran away after 7 days in excellent health. Another (no. 10) did the same thing 4 days after operation, without having had symptoms. Three were allowed to survive a long time; in two the remaining cortex was removed 217 and 232 days after the second operation (nos. 8 and 14); they died in 24 and 26 hours with symptoms of double adrenalectomy. The remaining eight were used in different experiments; 3 (nos. 3, 2 and 6) in the first week after operation, 1 (no. 11) in the second week, 3 (nos. 13, 15, and 16) in the third week, 1 (no. 12) a month after the second operation.

All the animals that survived had no abnormal symptoms; once the skin had healed and the hair grown, no difference could be found from normal dogs.

In five of these the pancreas was removed according to Witzel's technic, somewhat modified. Two survived 8 and 3 days, the sugar in the blood rising after operation from 0.7 per cent to 4 per cent and from 0.8 per cent to 3.63 per cent (Folin and Wu's method). Sugar in the urine reached 100 per cent (Fehling's method). In the second dog, a

Fig. 1. Left suprarenal gland, after operation. In the center scar left by surgical wound. Round it cortical tissue. No chromaphil cells are to be found.

small portion (100 μ × 100 μ × 1 mm.) of medulla was found; in the first dog not the slightest residue of chromaphil tissue was present. The 3 other dogs maintained, or slightly lowered their blood sugar. In one a small part of the pancreas was found. The other two died 6 and 24 hours after operation, certain surgical difficulties had been found, and shock had been produced.

In seven dogs, among those that had survived longest, the left adrenal was stained and searched for chromophil tissue, only in the one

already mentioned was medullary tissue found; the others had not the slightest portion left behind. The cortex was in good state, with numerous vacuolated cells (spongiocytes). A central scar remained, from which tracts of sclerosis branched out.

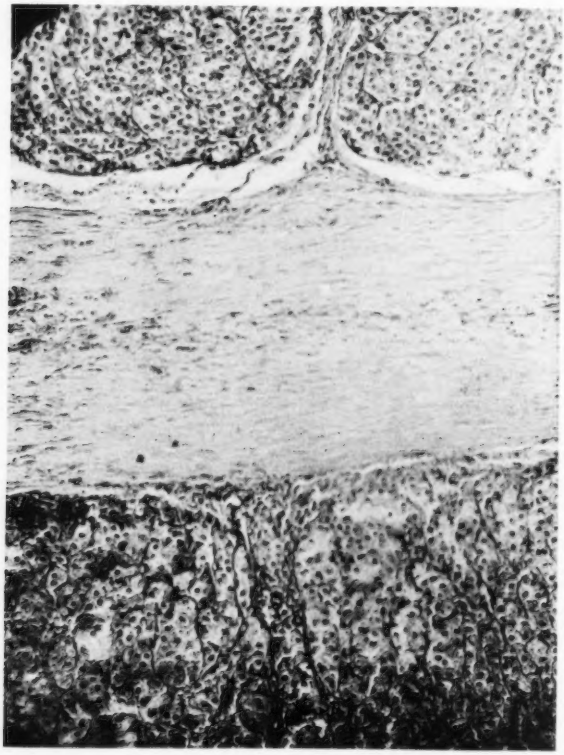

Fig. 2. Left adrenal gland after operation. Cortical tissue in direct contact with central scar. No chromaphil cells.

A second series of 9 dogs was operated in the same way as described, four of which died on the day following the second operation; the other five survived and a month after were in excellent health.

Two were depressed during a short time after the operation, later this symptom disappeared, and like the rest they ran about actively, were

affectionate and in all ways behaved as normal dogs. Weight increased in two, remained stationary in two, was lowered in one. Pulse-rate, breathing and temperature were found normal. Some had infections in the surgical wounds, but healed as is usual. In none was found abnormal pigmentation of skin or mucosae. Hair was in excellent state.

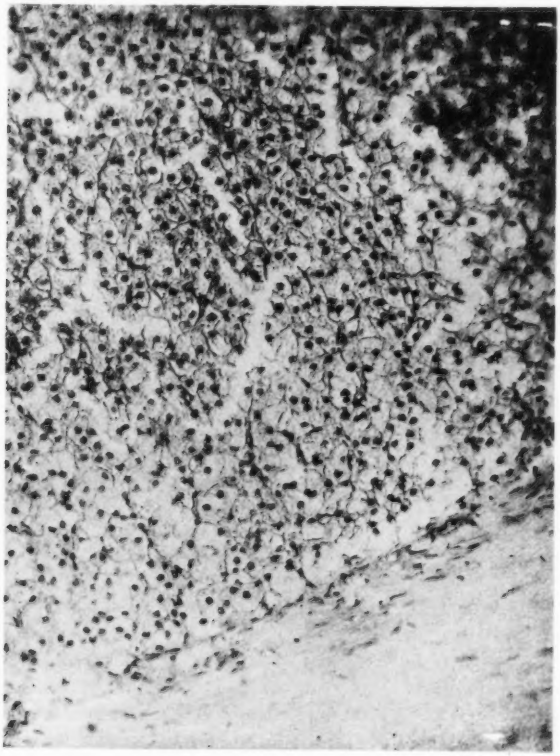

Fig. 3. Cortex cells on boundary of central scar. Typical spongiocytic aspect.

A month after the second operation blood pressure was measured in the femoral artery and adrenalin or pituitary extract injected. Blood pressure was found normal (115 mm. Hg. mean) and similar to that found in controls. Intravenous injection of adrenalin (Parke-Davis) 1:10,000,000, 1:1,000,000, 1:100,000, and 1:50,000 had the same effect in the 5 operated dogs as in controls.

Injection of 0.5 cc. "Infundin" (20 per cent pituitary extract) in the jugular vein produced the same effect as in controls.

Application to the eye of 1:100 solution of cocain chlorhydrate, 1:200 atropin sulphate and 1:1000 adrenalin produces mydriasis of same duration and degree as in normal animals.

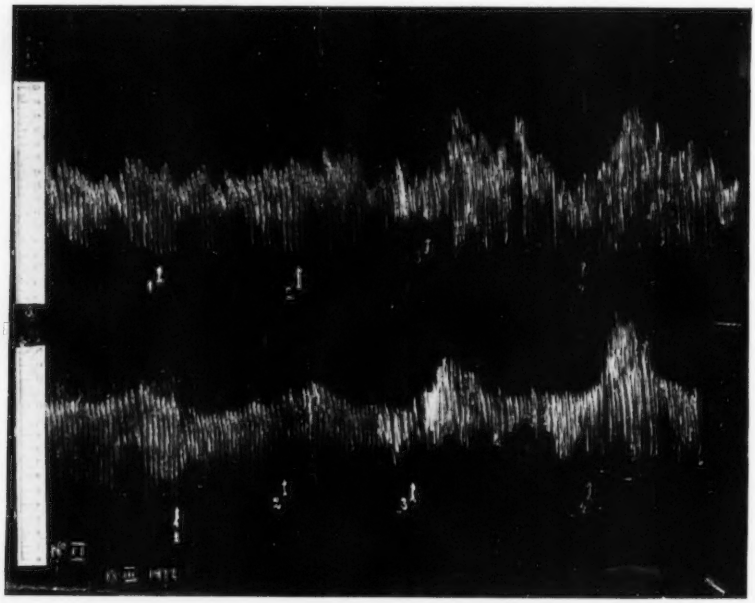

Fig. 4. Blood-pressure curve. *1*, Injection 1 cc. adrenalin 1:10,000,000; *2*, 1 cc. adrenalin 1:1,000,000; *3*, 1 cc. adrenalin 1:100,000; *4*, 1 cc. adrenalin 1:50,000.

Blood analysis (Folin and Wu's method) revealed normal quantities of

Glucose per 100 cc	0.123	0.132	0.11	0.098
Non-proteid N. per 100 cc	0.027	0.04	0.03	0.028
Urea per 100 cc	0.012	0.018	0.015	0.012
Sodium chloride per 100 cc	0.435	0.44	0.445	0.438

Two had gaseous gangrene in wounds made for blood-pressure estimation, two bit through ligatures during the night and died of hemorrhage, the other was killed. Necropsy revealed nothing abnormal.

Accessory glands were not found. The right adrenal had been totally removed. The left was stained and no chromophil tissue could be found. Its centre was occupied by a scar. Cortex cells had a typical spongiocytic aspect.

Discussion. Our experiments lead to the same conclusions as Biedl's and Wheeler and Vincent's, but the constancy of results obtained when

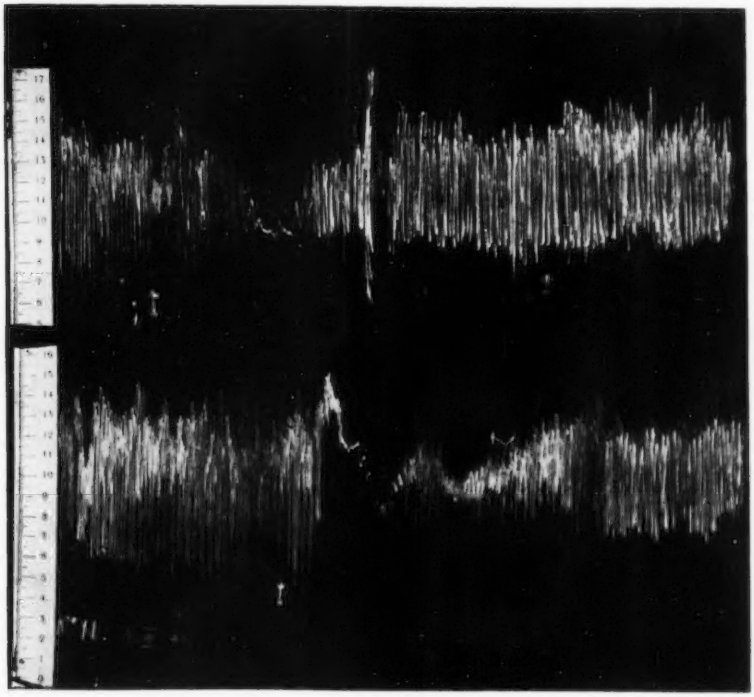

Fig. 5. Blood-pressure curve. Injection of 0.5 "Infundin" (20 per cent pituitary extract).

following our technic and the number of our experiments remove all doubts that may have remained.

Apparently contradictory evidence obtained by other authors can be explained by analyzing their experiments.

Cristiani's work can not be taken into account, as rats normally survive double adrenalectomy—60 per cent to 80 per cent according to our experiments.

Vassale and Zanfrognini possibly did severe damage to the cortex as happened in some of our own experiments. The fatal results of removal of one adrenal and abdominal paraganglia must be explained by some fault in technic as it is commonly known that one adrenal is amply sufficient to maintain life. Accessory chromophil tissue though abundant in dogs is not of great importance, quantitatively considered. Swale Vincent remarks that though in this species it is more abundant than in others double adrenalectomy is always fatal. On the other hand the accessory adrenals of rats, a species which survives double adrenalectomy, are made up of cortical tissue (Wiesel, 13).

Death brought about by injection of cytotoxic serums as in Levi della Vida's experiments, can not be allotted exclusively to specific alterations of a determined tissue, these serums contain heterogenetic antibodies, especially hemolysins (Abbot, 14), that are by no means harmless.

SUMMARY

The following facts are definitely established: *1*, Double adrenalectomy is fatal in most species, amongst them the dog. *2*, Removal of all chromophil tissue contained in these glands is perfectly harmless. *3*, Extirpation of inter-renal body (equivalent of cortex in Elasmobranchs) is followed by death. We may therefore conclude, *1*, The cortex is indispensable to life; it maintains its vital functions without the cooperation of the medulla. *2*, The chromophil tissue of the suprarenals is not necessary to life or to normal functions.

This does not mean that chromophil tissue is not indispensable to life. In our experiments only a part of this tissue is removed, and it must be remembered that relatively a greater portion of medullary tissue than of cortical tissue remains in the body after adrenalectomy. If chromophil tissue has vital functions, the extra-capsular portion is sufficient to maintain them, but up to now we have no evidence that these vital functions exist.

CONCLUSIONS

1. Dogs survive extirpation of all chromophil tissue contained in the adrenals, when the remaining cortex is in good state.

2. If the remaining cortex is removed, animals die as after double adrenalectomy.

3. Dogs without the adrenal medulla maintain their functions normally. Weight is not lost. Body temperature, pulse rate, blood pressure, breathing and the chemical composition of blood are normal.

4. No abnormal pigmentation or asthenia is observed.

5. Sympathetic irritability, as shown by injection of adrenalin or by pupillary reaction toward cocain, adrenalin and atropin, is normal.

6. The blood-pressure response to injection of pituitrin is normal.

7. Pancreatic diabetes can be established without secretion of adrenalin.

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BIOLOGICAL FOOD TESTS

I. Vitamin A in some Citrus Fruit Products

AGNES FAY MORGAN

From the Laboratory of Household Science, University of California, Berkeley

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Although the technique used in the determination of the vitamin content of available foodstuffs is not as yet thoroughly standardized, apparently enough progress has been made in this direction to justify the undertaking of a definite examination of individual foods for those biological properties which are not measurable by chemical means. It has long been recognized that a large amount of investigation of this sort is desirable in order to make our records of the dietary values of all available foods sufficiently complete for intelligent selection in accord with nutritive needs. For whatever may be the eventual determination of the physiological rôle of the vitamins, or even of their chemical structure, there remains the practical necessity for comparisons as to quantitative effectiveness of the naturally occurring foods as sources of these vitamins.

The problem is not one which can be disposed of by analogy or by averages as can some of the chemical questions concerning foods, for every attempt in this direction so far made has met with serious objection and inexplicable exceptions. In this connection might be cited the promising hypothesis of Steenbock (1) concerning the probable occurrence of vitamin A along with the lipochromes, and the belief of McCollum (2) that the development of this vitamin is related to the biological function of the plant tissue. The notable exceptions to the former theory cited by Palmer, Kennedy and Kempster (3), by Drummond and Coward (4) and by Steenbock, Sell and Buell (5), are too well known to repeat, and the wide dissimilarities in vitamin content reported by Steenbock and Boutwell (6) for vegetable products of the same type invalidate to some degree the latter theory.

Only the painstaking study of each food product individually under constant and comparable conditions will serve the important purpose

set forth above. A considerable amount of work upon this problem has been done in many different laboratories, and fortunately much of it has been done under fairly comparable conditions. It is clear, however, that the task has only been begun and that many investigators working with the same methods must still contribute to this end. Numerous stumbling blocks in the way of this accumulation of reliable data will occur even under the most carefully planned attempt at production of reproducible conditions. Some of these are connected with the varying composition of food samples examined, possible effects of soil, irrigation, proportion of sunshine, time of harvesting, length and conditions of storage, time and temperature relations in drying, cooking or otherwise preserving the supply of the sample. Other differences will possibly result from variations in viability and rate of growth of the animals used, from differences in temperature conditions, caging, constancy of basal diet.

It was with lively consciousness of these limitations that the studies here reported were planned. The same processes were employed throughout all the experiments, and modifications introduced only where the nature of the material used made them necessary. It was particularly desired to obtain as accurate records as possible of the total food intake of the animals upon the various diets in the hope of establishing the relation of appetite to the gravity of deficiency symptoms noted. Such a relation has been observed in the case of vitamin B, but has not been established for vitamin A. Indeed, Aron (7) has recently published data to show that the appetite may even be normal throughout a severe condition of vitamin A deficiency. Most of the cases reported in the present study indicate the same condition.

The plan of these biological tests includes, *a*, feeding experiments to determine the nutritive value of the nitrogen compounds of various foods, using as subjects rats and mice; *b*, the determination of the minimum daily dosage which protects young rats against vitamin B deficiency, or which restores normal conditions in young rats failing from such deficiency; *c*, the same determinations for vitamin A; *d*, the minimum dosage which restores to a normal condition guinea pigs made scorbutic by vitamin C deficient diet.

In certain cases some of these properties have already been determined by other investigators, and in such cases the known factors were not always redetermined. It is recognized, however, that a complete report on all these points made by the same methods in the same laboratory is desirable, even though some of the information has previously been collected under other conditions.

Since only vitamin A determinations are reported in this paper, the detail of method used for that type of investigation is presented at this time.

EXPERIMENTAL METHODS FOR DETERMINATION OF VITAMIN A. In these studies there were used the usual methods of caging, feeding and caring for the experimental animals which have been frequently described by other workers in this field.

The rats were the albino and mixed albino varieties secured by breeding mature healthy stock in our own colony. The experimental animals were weaned at 3 to 4 weeks of age when their body weight did not exceed 50 grams. Each animal was placed in a separate cage upon a wire gauze covering the absorbent paper in the bottom of a granite pan. Porcelain feeding cups and glasses for water were adjusted in each cage. Fresh water, new portions of basal diet, and the added food being tested, were given daily. All food residues were collected daily and weighed. Each animal was weighed either once or twice a week, and the food reserve glasses filled with 100 grams of basal diet whenever necessary. In this way an exact record of food intake was obtained with very little labor.

Basal diet. The basal diet used in all cases was made up as follows:

Casein	18
Agar	2
Salts	4
Dextrin	56
Fat	20

Preparation of casein. The casein, precipitated with lactic acid was obtained in large quantity in a finely ground dry condition from a large creamery company. This was at first further prepared by extracting with three successive portions of absolute alcohol, and then with ether according to the method proposed by Osborne and Mendel (8).

This method was later discarded, however, in favor of a modification of that proposed by Coward and Drummond (9). In this process the casein is baked in flat pans in a thin layer for 24 hours at 130°C. with frequent stirring to provide the maximum oxidation. The casein turns dark brown in color during this process, but is apparently not injured in flavor nor in protein character, for numerous animals have been reared and carried through an average span of life upon it as practically the sole source of nitrogen.

A few comparisons were made upon the relative efficiency of the two processes described above as a means of destroying traces of vitamin

in the casein, and the oxidation method discovered to be invariably the more effective. Since the labor and expense involved in this method is very much less also than in the alcohol extraction method the former was adopted for all subsequent work.

Preparation of dextrin. Cornstarch was heated at 170° to 180°C. in the same manner as the casein with frequent stirring for 5 to 7 hours. This resulted in the formation of an even light brown dextrin from which all vitamin traces were absent.

Salt mixture. The salt mixture proposed by Osborne and Mendel (10) was used,—being made from as pure salts and acids as could be obtained.

Agar. The purified shredded agar usually marketed for bacteriological work was used.

Fat. When a basal diet free from vitamin A was desired, the hydrogenated fat, crisco, was employed. This fat was adopted because it is fairly uniform in quality, and because the high temperature employed in hydrogenation makes it probable that very little vitamin from the cottonseed oil used remains active. The work of Drummond (11) on this point suggested the use of crisco.

Whenever a diet sufficient as to vitamin A was desired, 10 parts of butter fat, separated in the usual way, were substituted for 10 parts of the crisco. No other change in the basal diet was made.

Vitamin dosage. When the vitamin A content of a food was to be tested, vitamin B was provided by the addition of 0.3 gram of dried brewery yeast to the daily ration. This amount was found to yield full growth in rats from the time of weaning to the age of 6 months. The yeast was placed on top of the basal diet in the food cup and was immediately and completely eaten by the rats.

The substance to be tested was likewise weighed out daily and placed on top of the basal diet. Very little difficulty in inducing the rats to consume these portions was experienced with any of the foods here described.

Method of testing. Both of the currently used methods of testing quantitatively the vitamin content of foods were employed.

In the first, called curative, rats 3 to 4 weeks old were placed on vitamin A free or vitamin B free diet, the other vitamin being supplied in each case, and kept thus till they showed definite flattening of the weight curves and such other bodily defects as are characteristic of these deficient diets. In the case of vitamin A-free diets all rats employed regularly showed definite eye disease and stationary or falling

weight within 3 to 5 weeks. There were no exceptions in the course of all the studies carried out, in which there were used some four hundred animals. The food to be tested was then given in known amounts daily, and judged to be of value in proportion to the speed of cure of eye condition and resumption of body growth upon minimal dosage. The results of vitamin B deficiency were not as clear-cut as those described for vitamin A, but the flattening of the weight curve was attained in much the same period, and upon prolonged vitamin B deficiency the characteristic weakening of the hind legs developed with increasing emaciation and death. None of these animals ever showed traces of eye disease even though they were caged in close proximity to animals suffering from extremely severe cases.

Respiratory infections were common among animals upon vitamin A deficient diet as has been noted by McCollum (12) and others, but these symptoms were not observed in neighboring control or vitamin B deficient rats.

The second method of testing for vitamin values was of preventive character. The animals were fed minimal doses of the food in addition to the basal diet from the time of weaning, and the values computed from the size of dose necessary to protect against characteristic disease and to produce a normal rate of growth. This method was not found to give as sharp distinction to the value of different quantities employed as was the curative method. At least for vitamin A work there can be little doubt that the latter method is the more satisfactory.

In all cases one or more animals from each litter used were placed on the basal diet containing butter and in addition were given 0.3 gram dried brewery yeast daily. These animals were found to grow satisfactorily in all cases and were looked upon as furnishing curves of normal growth for the litter. In a number of cases, rats which had been reduced to subnormal condition by feeding deficient diets were retrieved with codliver oil, butter fat or yeast in an attempt to establish standard curves of recovery. Such curves are perhaps more helpful to use in judging the value of unknown foods than the uninterrupted curves of the usual normal animal.

Results with certain citrus fruit products. A survey of the literature dealing with the nutritive value of the citrus fruits, namely, the orange, the lemon and the grape-fruit, indicated that considerable information is available with regard to the antiscorbutic properties of these fruits, but very little as to any other vitamins which might be present. An attempt was made, therefore, at evaluation of the juice,

peel and oil of these fruits with regard to vitamins A and B. It was assumed that as sources of nitrogen these products were not of special interest, and for the time being nothing was done with their nitrogen values.

Vitamin A in citrus fruits. Very little attention has been directed heretofore to the possible value of citrus fruits as carriers of vitamin A. A generalization on the subject appears in a report by Osborne and Mendal (13) in which the vitamin B content of orange juice and of the inner orange peel was discussed. In a later preliminary statement (16), however, they give certain approximate figures for possible presence of this substance in orange juice. A preliminary statement by E. Cooper (14) points out the presence of vitamin A in citrus fruit peel, but again no protocols have been submitted.

A study was undertaken, therefore, of the value as to vitamin A of the peel, juice and oil, from oranges, lemons and grapefruit. There are reported here results as to orange peel, orange juice, orange oil, lemon peel, lemon oil and grapefruit peel.

Orange oil as source of vitamin A. Four preparations of the oil of orange peel were tested for vitamin A content. The first of these was an ether extract, 1.8 per cent of dry weight of the peel, which was a very dark orange in color, fragrant and fairly volatile. The second and third samples were rather light orange oils of less fragrance and greater astringency obtained as an oil layer separated by centrifugation from the juice resulting from cold pressure of the whole orange. These products were obtained from the research laboratory of the California Fruit Growers' Exchange.

The second sample was made from fresh ripe navel oranges, picked early in the season, by cold compression and separation of the oil from the pulp by centrifugation. The third sample was prepared in the same way from ripe Valencia oranges which had hung for some weeks upon the trees and which were somewhat desiccated.

The fourth oil, obtained from the laboratory of the California Citrus Fruit Juice Company, was practically colorless and odorless. This oil was obtained by steam distillation of the orange peel.

The first of these oils, the ether extract, was found to be a surprisingly rich source of vitamin A. It was effective in doses of as little as 0.1 gram daily in curing the most advanced cases of the eye disease characteristic of vitamin A deficiency and in promoting the resumption of growth in animals stunted by the same deficiency. A comparison of this ether extract with codliver oil administered in similar amounts

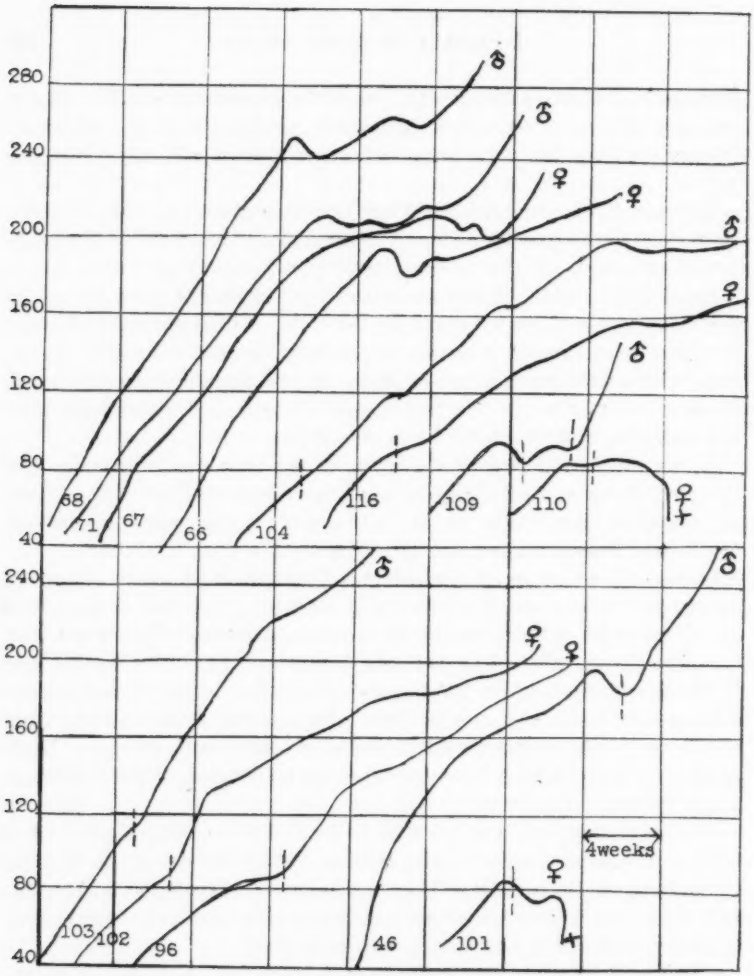

Chart 1. Illustrative growth curves of rats fed citrus fruit products as source of vitamin A.

Basal diet and 0.3 gram dried brewery yeast daily given in each case in addition to source of vitamin A, administration of which was begun at dotted line in curative experiments.

Rats 68 and 66 fed 0.2 gram codliver oil; 67 and 71, 0.1 and 0.2 gram orange oil. Rats 109 and 110 fed lemon oil. At second dotted line 109 was given instead 5 grams dried skim milk.

Rats 104 and 116 fed 1 gram lemon peel; 101, 1 gram grapefruit peel; 96 and 46, 1 gram orange peel.

Rats 103 and 102 fed 12 cc. orange juice.

and under identical conditions as to basal diet, caging, animals, etc., showed the orange oil used to be somewhat superior in vitamin A value. Four animals were used in each series and the feeding carried on for 23 weeks in each case, until the female rats had attained an average weight of 200 grams and the males a weight of 250 grams.

TABLE 1

Weekly food intake of rats with and without orange oil

RAT 67	RAT 44	RAT 70	RAT 71	RAT 72	RAT 117
Basal diet and 0.1 gm. ether extract of orange peel	Basal diet	Basal diet and 0.1 gm. of ether extract of orange peel	Basal diet and 0.1 gm. of ether extract of orange peel	Basal diet and 0.1 gm. of ether extract of orange peel	Basal diet
60	100	64	74	65	38
43	100	58	60	58	62
47	112	65	64	63	65
30	114	56	64	60	24
15	88	89	63	90	30
85	112	70	75	77	
78	78	70	90	74	0.1 gm. ether extract orange peel
50	63	50	50	60	
80	54	100	100	100	50
90	32	75	70	75	74
52	36	61	73	50	70
55	36	40	70	65	80
Basal diet and 0.2 gm. cold pressed orange oil	0.1 gm. ether extract orange peel				35
	105				35
100	100				58
85	100	Basal diet and 0.2 gm. cold pressed orange oil			
70	152	75	100	100	89
77	100	70	58	60	
77	100	58	50	70	
50		40	50	65	
70		50	50	50	
		50	59	50	
		50	50	50	

The second orange oil, that obtained by cold pressure, from young navel oranges, was also a valuable carrier of vitamin A, although not in as large degree as was the ether extract of the peel. A larger quantity was necessary, 0.2 gram, to maintain normal weight relations in animals to full maturity. The same type of comparison was made with cod-liver oil, carried on for the same length of time, and the result showed

the cold pressed orange oil to be of practically the same value as a carrier of vitamin A as was the codliver oil used. Within 2 weeks of the beginning of administration, normal growth and condition of the eyes were attained.

The third orange oil, that obtained by cold pressure from over-ripe, somewhat desiccated Valencia oranges, was ineffective in any dosage which could be administered. Eight young rats were placed upon vitamin A-free basal diet with the usual 0.3 gram of dried brewery yeast, and upon development of eye disease and stunting after 28 days, were given increasing quantities of the orange oil. After 6 weeks four of these animals were dead and of the four surviving only one weighed more than 80 grams and had recovered a normal condition of the eyes.

It is evident that oxidation or desiccation of the peel of the fruit through long standing upon the trees had practically completely destroyed the vitamin A present in generous amount in the young fruit. The difference in species of the oranges used may also have had some bearing upon this change.

The fourth orange oil, that obtained by steam distillation, was without value as a source of vitamin A. The animals used died or were moribund within 4 weeks after decline and dosage had begun.

It is of interest to note the concurrence of vitamin A content and depth of color in these oils in connection with the lipochrome theory of Steenbock (1). No attempt was made to establish a quantitative relation between the color and the potency of these oils, nor was any chemical analysis of the oils carried out. It is evident, however, that considerable difference in chemical constituents, as well as in vitamin potency, would result from the varying methods of extraction used.

Orange peel as source of vitamin A: Dried orange peel, from which the inner white coat had been removed, was finely ground and administered in doses of $\frac{1}{2}$ gram and 1 gram to young rats which had declined from lack of vitamin A. In all cases a remarkable recovery ensued. The effective dose seems to lie between $\frac{1}{2}$ and 1 gram daily. Seven rats, all males except one, were used in this study. All of these animals showed severe eye inflammation before the curative dose of orange peel was given. In most cases the eyes had become normal within a week afterward. The feeding was continued for 23 weeks till full mature weight was attained, and excellent appearance of nutrition in coat, tail, eyes and teeth maintained throughout the period of observation. These findings are in accord with results indicated in a preliminary statement recently made by E. Cooper (14).

No invariable relation was noted between the total food intake and the administration of the food containing vitamin A. The total weekly consumption of basal diet is given in table 2. This is, of course, not the case with animals deprived of vitamin B, which invariably acts as an appetite stimulant.

Experiments upon the retention of its vitamin A by orange peel which had been cooked in a thick syrup are in progress.

TABLE 2

Weekly food intake of rats with and without orange peel

RAT 42	RAT 43	RAT 46	RAT 47	RAT 51	RAT 53
Basal diet	Basal diet	Basal diet	Basal diet	Basal diet	Basal diet
100	100	70	78	70	77
88	77	73	86	80	91
77	80	78	100	83	114
58	100	88	40	73	88
58	60	88	40	80	60
70		88	45	88	58
60	1 gm. orange peel	100		40	
		50	1 gm. orange peel		1 gm. orange peel
1 gm. orange peel	75	90		1 gm. orange peel	
	70	89	100		77
80	72	55	100	100	80
79	73		50	84	100
72	100	1 gm. orange peel	100	95	85
35	95		100	60	80
75	78	74	100	100	100
96	100	85	92	75	65
98	88	68	83	97	60
165	52	100	100	100	60
100	80	130	144	85	84
100	100	100	90		
	80	100	85		
	70				

Orange juice as source of vitamin A. A concentrated orange juice, made by evaporation of the fresh juice under reduced pressure to one-sixth the original volume,[1] was used as a means of restoring animals which had begun to fail from vitamin A-deficient diet. All of these animals showed particularly advanced cases of eye disease, but all

[1] This concentrated juice was kindly supplied by the California Citrus Fruit Juice Company, of San Francisco.

treated by orange juice became practically normal within 10 days. A curious exception occurred in the case of a control animal of this litter which was given codliver oil as standard restorative. This animal had a particularly malignant condition in both eyes, with corneal ulcers, which were never entirely removed. One eye, otherwise normal in

TABLE 3

Weekly food intake of rats with and without orange juice

RAT 98	RAT 99	RAT 100	RAT 102	RAT 103
Basal diet	Basal diet	Basal diet	Basal diet	Basal diet
80	80	80	65	68
50	60	45	45	67
30	30	43	30	14
40	40	37	41	34
0.1 gm. cod-liver oil	12 cc. orange juice	12 cc. orange juice	12 cc. orange juice	12 cc. orange juice
70	60	73	60	85
66	63	70	62	85
42	52	56	62	70
68			47	100
65	18 cc. orange juice	18 cc. orange juice	78	100
92				98
50	90	100	30 cc. orange juice	100
50	80	100		100
50	75	105	60	109
50	45	70	50	100
45	45	70	50	100
45	44	70	50	100
45	50	50	42	
50	50	50	45	
50	50	70	60	
50	56	70	50	
	56	50	50	
	60	60	50	
			58	

appearance, after 3 weeks' administration of 0.1 gram codliver oil daily, was considerably enlarged, the other, even after 5 months, showed a protruding corneal growth. This rat grew at a normal rate after codliver oil dosage began.

Two cubic centimeters of the concentrated juice, equivalent to 12 cc. of the fresh substance, was the minimum amount which effected

the cures described above. This quantity is similar to that found by Osborne and Mendel (13) to be sufficient for provision of water-soluble B and a little more than that (10 cc.) which they found (16) adequate for vitamin A. The experience of Hess, McCann and Pappenheimer (15) in their investigation of the relation of the fat-soluble vitamin to the development of rickets, bears out these findings in that the rats to which 0.5 cc. orange juice daily was given developed eye disease less regularly and declined in body weight less than animals having similar treatment but without orange juice. It is evident that in these cases the quantity of orange juice provided was too small for complete protection.

Lemon oil as source of vitamin A. Oil extracted from whole fresh young lemons in the same manner as that described for the navel orange cold pressed oil was supplied by the California Fruit Growers' Exchange Laboratory, and was tested by the curative method. All of the animals used had developed an extremely severe condition of the eyes, as well as falling weight line when the oil administration was begun. The amount given was raised gradually from 0.1 to 0.3 gram, but the animals continued to fail in all cases, two of them dying, and two being recovered from a moribund condition by means of other vitamin carriers. The interesting curative action of skim milk powder used for rat 109 will be discussed in another connection. It may be stated here, however, that upon this diet the animal eventually failed with return of the eye disease.

During the first few days that the lemon oil was given, the rats showed some indisposition to eat it, but this was soon overcome and a normal quantity of basal diet, as well as the full dosage of lemon oil, was consumed. These figures are shown in table 4. The failure cannot be assigned, therefore, to the animals' dislike for the flavor of the lemon oil nor, indeed, to a failure to consume enough basal diet.

The lemon oil used was practically colorless and exhibited much the same flavor and odor as the usual commercial lemon extract. The interesting contrast of these results with those obtained from orange oil obtained in the same way, cannot at present be explained.

Experiments are in progress upon the vitamin A value of lemon juice and of the ether extract of lemon peel.

Lemon peel as source of vitamin A. Dried lemon peel, from which the inner white skin had been removed as completely as possible, was used for curative action upon rats which had been reduced to disturbed weight relations and which were suffering from a serious form of the

eye disease characteristic of vitamin A deficiency. In all cases 1 gram of the dried peel was given and improvement of eye condition and resumption of growth resulted. The animals could not be induced to consume appreciably larger amounts of the peel, so that it was impossible to determine whether larger dosage would induce more rapid curative action. One of the animals, rat 108, retained one closed and slightly inflamed eye throughout the experiment. The others cleared up the eye condition, but exhibited somewhat subnormal weight curves.

TABLE 4

Weekly food intake of rats with and without lemon oil

RAT 94	RAT 105	RAT 109	RAT 110
Basal diet	Basal diet	Basal diet	Basal diet
88	64	75	75
40	50	50	50
38	69	65	84
63	35	15	20
25	0.1 gm. lemon oil	0.1 gm. lemon oil	0.1 gm. lemon oil
0.1 gm. lemon oil	50	30	10
45	0.2 gm. lemon oil	50	0.2 gm. lemon oil
60	72	0.2 gm. lemon oil	60
0.2 gm. lemon oil	40	70	42
47	1 gm. artichoke	50	Dead
0.3 gm. lemon oil	58	5 gm. skim milk	
42	56	50	
45	56	35	
45	30	35	
55	50	70	
Dead	70	58	
	70	50	
		50	

It is evident that the lemon peel is considerably less effective as a carrier of vitamin A than similarly treated orange peel. As may be seen from table 5, the failure cannot be ascribed to a dislike of the lemon peel by the rats nor to consumption of inadequate amounts of basal diet.

Grapefruit peel as source of vitamin A. Grapefruit peel prepared in the same manner as was the orange and lemon peel was tested in 1 gram

doses for curative effect upon two animals in the same precarious condition as to growth and eye disease as was used with the lemon peel and orange peel. Both animals failed continuously, one of them dying within 2 weeks, and the other showing stationary weight and greatly increased severity of eye condition, till recovered by administration of orange peel. Although only two rats were used, the results were

TABLE 5

Weekly food intake of rats with and without lemon peel

RAT 104	RAT 108	RAT 116	RAT 106
Basal diet	Basal diet	Basal diet	Basal diet
50	65	75	60
50	50	75	50
72	89	72	78
30	15	47	25
1 gm. lemon peel	1 gm. lemon peel	1 gm. lemon peel	1 gm. butter
58	44	48	40
65	50	60	75
82	55	75	68
70	50	70	58
50	40	70	50
70	50	58	50
70	42	58	50
58	50	50	50
58	58	58	50
60	58	58	45
60	70	50	48
70	42	50	58
77	58	50	58
50	58	50	70
56	56	70	70
63	58	50	50

decisive when viewed side by side with similar treatment with lemon and orange peel.

The animals ate the grapefruit peel less completely than the orange or lemon peel, but the major portion of the daily dose was consumed in both cases.

Similar experiments upon ether extract of grapefruit peel, cold pressed grapefruit oil and grapefruit juice are in progress.

These negative findings as to vitamin A in grapefruit peel are not in accord with positive results indicated in the preliminary statement by E. Cooper (14).

TABLE 6

Weekly food intake with and without grapefruit peel

RAT 96	RAT 101
Basal diet	Basal diet
77	60
66	68
35	62
32	40
40	
1 gm. grapefruit peel	1 gm. grapefruit peel
	23
50	Dead
50	
1 gm. orange peel	
63	
75	
80	
65	
50	
50	
55	
55	
70	
70	
70	

SUMMARY

A report is given as to methods in use in this laboratory for the determination of the vitamin A content of foods.

By these methods the vitamin A content of orange oil, extracted by ether or by cold pressure, was found to be not far different from that of fresh commercial codliver oil, as indicated both by promotion of normal growth and by curative action in cases of vitamin A deficiency.

A third sample of orange oil obtained by cold pressure from ripe Valencia oranges which had remained several weeks after reaching maturity, upon the trees, was practically devoid of vitamin A.

A fourth sample of orange oil obtained by steam distillation, was of no value in this regard. These four orange oils showed depth of orange color in the same order as their vitamin A efficiency.

Dried outer orange peel in doses of 0.5 to 1 gram is effective in restoring normal conditions in animals showing extreme emaciation, eye disease and other indications of vitamin A deficiency.

Orange juice, given in a concentrated syrupy form was effective as a curative source of vitamin A in doses corresponding to 12 to 30 cc. of fresh juice.

Dried outer lemon peel in doses of 1 gram was effective as a curative source of vitamin A, but less stimulating to growth and removal of eye symptoms than the orange peel.

Lemon oil obtained by cold pressure was found to be ineffective as a source of vitamin A under these conditions.

Dried outer grapefruit peel was likewise found to be ineffective under these conditions.

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BIOLOGICAL FOOD TESTS

II. Vitamin A in Skim Milk

AGNES FAY MORGAN

From the Laboratory of Household Science, University of California, Berkeley

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The distribution of vitamin A between the watery and fatty portions of cow's milk has not been satisfactorily established in spite of the considerable interest of both practical and theoretical nature involved in the problem. The statement is ascribed to McCollum (1) that this vitamin is thirty times more soluble in fat than in water, and that there would, therefore, be an approximately equal division of it between butter-fat and skim milk. No experimental basis for this statement is quoted. In a brief statement by McCollum, Simmonds and Steenbock (2) this idea is put forward with reference to feeding experiments in which butter-fat washed with twenty successive portions of water failed to maintain normal growth in animals fed on a diet adequate except for vitamin A. These authors assume, therefore, that vitamin A is water soluble to a considerable degree. The details of these experiments have not been published so far as is known to the writer. Sherman, MacLeod and Kramer (3) in another preliminary statement have described, without detail, experiments in which successful growth of young rats was secured by the use of dried skim milk as sole source of vitamin A, trebling their body weights in 3 months and remaining free from eye disease and in good general condition. They were also able to restore to normal, by use of dried skim milk, rats which had been brought to the typical condition of eye disease and declining weight due to deficiency of fat-soluble vitamin in their food. Quantitative relations were not established, however, nor have the details of these experiments yet appeared.

It seemed of interest, therefore, to attempt to find a more definite answer to the question raised by these statements. The plan adopted in this laboratory was that of both prophylactic and curative methods of feeding to prevent or remove vitamin A deficiency symptoms, and

to carry on the feeding over a period of at least 4 months after the administration of the skimmed milk was begun.

EXPERIMENTAL METHODS. The same methods described previously (4) were used, and one type of skim milk powder adopted for the investigation. This is a skimmed milk powder prepared by the Central California Creameries by a spray process, and placed on the market in large well-standardized quantity, so that an excellent constancy of composition is maintained.

Prophylactic experiments. Fourteen young rats were placed on the usual vitamin A-free basal diet, supplemented by dried skimmed milk in varying proportions. Eight of these animals received a diet in which the skim milk formed 15 per cent of the dry weight from the time of weaning for periods varying from 8 to 14 weeks. In three cases, rats 2, 5 and 6, the proportion of skimmed milk was then raised to 25 per cent and kept thus for 4 to 7 weeks. This was followed by a period of 6 to 14 weeks during which 5 grams daily of the dried skimmed milk was given in addition to the basal diet. Three of the rats, 17, 18, 20, had the 5 gram dried milk addition from the beginning. Two, nos. 15 and 2B, had the 25 per cent skimmed milk diet from the beginning.

None of these animals grew at a normal rate, all of them developed respiratory infections, several showed a progressively serious eye condition, and three of those which showed the most rapid early gains in weight died under the treatment. The detail of the response to changes in diet is given in table 1.

Curative experiments. Eight young rats were placed on the vitamin A-free basal diet after weaning and kept thus to the stage of declining weight and eye disease typical of vitamin A deficiency. During this period 0.3 to 0.5 gram of dried brewery yeast was given daily, separately from the basa ldiet, to provide the necessary vitamin B. As soon as decline began, dried skimmed milk in daily portions of 5 grams, mixed with 20 per cent of its weight of crisco, was given in addition to the basal diet. In all cases an immediate improvement was noted in eye condition, weight increase and general state of nutrition. This impetus to renewed growth and health continued for varying periods, 17 weeks being the longest and 3 weeks the shortest observed. Where the typical eye inflammation was present at the time of skimmed milk dosage, an immediate improvement occurred, sometimes apparently to normal, but this condition in all cases proved temporary, with eventual recurrence and malignant progress of the disease, ending in death unless butter-fat or some other source of vitamin A were added to the diet. The detail of response to diets is given in table 2.

TABLE 1

Dried skimmed milk as prophylactic for vitamin A deficiency

RAT	DIET AFTER WEANING	LENGTH OF PERIOD	CONDITION AT END OF PERIOD	NEW DIET	LENGTH OF PERIOD	CONDITION	NEW DIET	LENGTH OF PERIOD	CONDITION
		weeks			*weeks*			*weeks*	
2	15 per cent skim milk	12	Weight declining	25 per cent skim milk	4	Stationary weight	5 gm. dry skim milk and basal	11	Stationary weight
5	15 per cent skim milk	8	Weight declining	25 per cent skim milk	7	Stationary weight	5 gm. dry skim milk and basal	14	Stationary weight
6	15 per cent skim milk	10	Weight declining; eye symptoms	25 per cent skim milk	4	Weight stationary; eye symptoms	5 gm. dry skim milk and basal	6	Stationary weight; eyes sore
7	15 per cent skim milk	14	Weight declining	5 gm. dry skim milk and basal	6	Weight stationary	1 gm. butter fat added	4	Rapid gain in weight
8	15 per cent skim milk	14	Weight declining	5 gm. dry skim milk and basal	3	Dead			
9	15 per cent skim milk	14	Weight declining	5 gm. dry skim milk and basal	14	Stationary weight			
10	15 per cent skim milk	14	Weight declining	5 gm. dry skim milk and basal	14	Stationary weight			
29	15 per cent skim milk	14	Weight declining	5 gm. dry skim milk and basal	6	Stationary weight	1 gm butter fat added	10	Rapid gain in weight
15	25 per cent skim milk	12	Dead						

2 B	25 per cent skim milk	16	Dead						
17	Basal diet and 5 gm. dry skim milk	13	Declining weight	0.1 gm. cod-liver oil and basal	8	Normal growth			
18	Basal diet and 5 gm. dry skim milk	14	Declining weight	1 gm. butter fat and basal	9	Normal growth			
20	Basal diet and 5 gm. dry skim milk	13	Declining weight	0.1 gm. cod-liver oil and basal	10	Normal growth			
23	Basal diet and 5 gm. dry skim milk	17	Declining weight	0.5 gm. butter fat and basal	7	Normal growth			

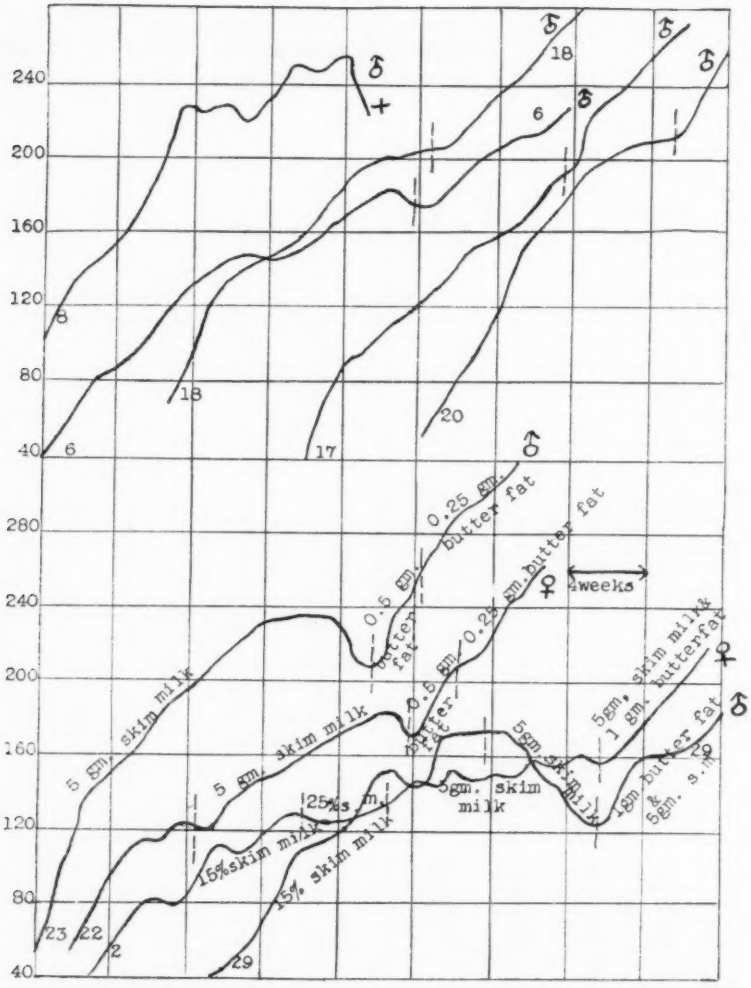

Chart 1. Illustrative growth curves of rats fed dried skim milk as source of vitamin A.

Basal diet and 0.3 gram dried brewery yeast daily given in each case in addition to source of vitamin A.

Dried skim milk, 5 grams, given 17, 18, 20 to dotted line then 0.1 gram codliver oil to 17 and 20, and 1 gram butter fat to 18.

Rats 6 and 8 given 15 per cent skim milk ration. At dotted line 6 was given in addition 1 gram butter fat daily.

Curves of rats 22 and 23 show comparison of 5 grams dried skim milk with 0.5 gram and 0.25 gram butter fat as source of vitamin A.

Curves of rats 6, 2 and 29 illustrate non-toxic effects of skim milk.

TABLE 2

Dried skimmed milk as curative of vitamin A deficiency

RAT	DIET AFTER WEANING	LENGTH OF PERIOD	CONDITION AT END OF PERIOD	NEW DIET	LENGTH OF PERIOD	CONDITION	NEW DIET	LENGTH OF PERIOD	CONDITION
		weeks			*weeks*			*weeks*	
11	Basal diet and 0.3 gm. yeast	13	Inflamed eyes; falling weight	Add 5 gm. dry skim milk	3	Same	1 gm. butter fat in-instead of skim milk	5	Normal
12	Basal diet and 0.3 gm. yeast	13	Inflamed eyes; falling weight	Add 5 gm. dry skim milk	3	Dead			
14	Basal diet and 0.3 gm. yeast	13	Inflamed eyes; falling weight	Add 5 gm. dry skim milk	3	Dead			
1 B	Basal diet and 0.3 gm. yeast	13	Inflamed eyes; falling weight	Add 5 gm. dry skim milk	4	Dead			
16	Basal diet and 0.3 gm. yeast	6	Inflamed eyes; falling weight	Add 5 gm. dry skim milk	8	Falling weight	1 gm. butter fat instead of skim milk	7	Normal
19	Basal diet and 0.3 gm. yeast	5	Inflamed eyes; falling weight	Add 5 gm. dry skim milk	5	Dead			
22	Basal diet and 0.3 gm. yeast	6	Inflamed eyes; falling weight	Add 5 gm. dry skim milk	11	Falling weight	0.5 gm. butter fat instead of skim milk	8	Normal
109	Basal diet and 0.3 gm. yeast	8	Inflamed eyes; falling weight	Add 5 gm. dry skim milk	17	Dead			

Comparison with butter-fat. In the case of two rats, an attempt was made to compare the quantity of vitamin A afforded by the 5-gram dose of dry skimmed milk with the minimum amount of butter-fat which could afford the same degree of protection. The amount of butter-fat was reduced from 0.5 to 0.25 gram but with no apparent diminution in the rate of growth and the well-being of the animals. These animals had previously declined in weight and exhibited eye symptoms upon the 5 gram dosage of skim milk.

It was found that 5 grams of dried skimmed milk daily was as large an addition as could be made to the diet without diarrheal complications and danger of deficiency due to too low intake of basal diet.

The administration of 0.25 gram and smaller additions of butter-fat is being continued over a longer period in a later series of experiments in the attempt at discovery of the point of failure in provision of vitamin A. The butter-fat used in these experiments was obtained from the same creameries which produced the dry skimmed milk, and in part from samples especially prepared from the same mixed whole milk.

The quantity of fat retained in the skimmed milk powder used was determined several times and found to be close to 0.7 per cent. The maximum claimed by the manufacturers is 0.9 per cent. Even on the basis of the latter figure the amount of butter-fat obtainable from the 5 gram dose used in these experiments did not exceed 45 milligrams daily.

It was thought possible that a toxic or growth-retarding substance might be present in the dried milk, as suggested by Mattill and Conklin (5), and that a diet made up so largely of this food might produce untoward results even independently of a deficiency in vitamin A.

In order to test the validity of this supposition, several animals which had begun to decline on the skim milk were given small quantities of butter in addition to the basal diet and skim milk. These rats, in all cases, grew normally. The number of experiments is not large enough to justify definite conclusions, but the indication seems to be that of deficiency in the skim milk rather than of the presence of an inhibiting substance or substances.

Diets used to show non toxic effect of skim milk: Normal growth on 1 gram butter-fat in addition to basal diet and 5 grams dried skim milk daily.

Basal diet

Casein	18
Salts	4
Agar	2
Crisco	20
Dextrin	56

15 per cent skim milk ration

Dried skim milk	15
Casein	12
Salts	3
Agar	1
Crisco	20
Dextrin	49

25 per cent skim milk ration

Dried skim milk	25
Casein	8
Salts	2
Agar	1
Crisco	20
Dextrin	44

Dried brewery yeast—0.3 gram daily in all cases

Composition dried skim milk

	per cent
Protein (N × 6.25)	34.6
Salts	6.4
Fat	0.7
Moisture	7.6
Carbohydrate	50.8

SUMMARY

1. It is shown that although dried skim milk, and presumably the fresh skim milk as well, is not altogether lacking in vitamin A, it is impossible to obtain normal growth over a prolonged period by the use of this substance alone.

2. An immediate and striking improvement was noted in most of the animals given the skim milk after decline from vitamin A deficiency. This was followed at varying intervals by an equally rapid decline, ending in death if the treatment was continued.

3. Successful growth on a diet containing both the skim milk and small amounts of butter-fat indicated that the eventual failure on the skim milk diet was probably not due to the presence of toxic or inhibiting substances in the skimmed milk.

4. Quantities as large as 5 grams of the dried skim milk were found to be less effective than 0.25 gram of butter-fat of the same origin. The proportion of vitamin A in dried skim milk and butter-fat would appear, therefore, to be less than 1 to 20, in fresh skim milk and butter-fat less than 1 to 220, and average whole milk should be more than 8 times more valuable as a source of vitamin A than the skim milk made from it.

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THE INFLUENCE OF SUGARS AND OTHER SUBSTANCES ON THE TOXIC EFFECTS OF INSULIN

E. C. NOBLE AND J. J. R. MACLEOD

From the Physiological Laboratory, University of Toronto

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Previous investigations from this laboratory (1) have shown that various characteristic symptoms supervene in rabbits some time after the injection of insulin, that these symptoms usually appear when the blood sugar has fallen to 0.045 per cent and that they can be rapidly removed by subcutaneous injections of a solution of glucose.

A problem which immediately presents itself for investigation is the causal relationship between the symptoms and the lowering of blood sugar. The symptoms give the impression that some substance having a highly irritative influence on the central nervous system has become developed, and the striking similarity of certain of the symptoms (convulsions) to those which develop temporarily, immediately after "spinning" a normal rabbit, suggests that the vestibular apparatus is being stimulated (Barany). It can scarcely be the case that this stimulus is afforded by a lowered percentage of glucose in the blood *per se*. It is more likely either that the hypoglycemia is related to the setting free of some toxic metabolic product which in the normal animal is directly or indirectly antidoted by a certain concentration of glucose, or that it causes some change in the chemical or physicochemical equilibrium within the cells, which results in a disturbance of their normal functions. One of the ways by which light can be thrown upon this question is to see whether other sugars than glucose have the power to remove the symptoms. If a given sugar fails to have this effect evidence is afforded not only that it cannot itself act like glucose but also that it is not converted, to any considerable extent at least, into glucose in the animal body.

Since all the common sugars with the exception of sucrose, reduce alkaline copper solutions, the blood sugar values as obtained by the Shaffer-Hartmann method will necessarily rise when the animals are injected with these materials, and in the small percentages existing in

blood there is no simple chemical method of ascertaining what portion, if any, of this rise may be due to glucose. On the other hand, if such an increase should occur when non-saccharine substances are injected, evidence of gluconeogenesis is afforded, especially if at the same time the convulsive symptoms are antidoted.

This part of our work is of a similar character to that of F. C. Mann and T. B. Magath (2) who found that the toxic symptoms which supervene some hours after the removal of the liver from the circulation in dogs, can be antidoted by the injection of glucose, and to a less degree of maltose, mannose, galactose and dextrose.

PROCEDURE. The experiments were all carried out on normal rabbits taken directly from the stock cages. A sample of blood was taken and a suitable dose of insulin injected subcutaneously. The rabbit was then left alone until it began to show symptoms, when a second sample of blood was immediately withdrawn. As soon as the convulsions became well marked, 2 grams per kilo or an equivalent amount of sugar or other material under investigation was injected subcutaneously in several places and the animal's behavior carefully observed while blood samples were withdrawn at suitable intervals. In certain cases the sugar solutions were given intraveneously, but since no difference could be detected in the results, this method was not used as a rule, because of the difficulty of applying it during the convulsive seizures.

RESULTS. Since glucose was the standard used in relieving convulsions and was the material with which the effects of other substances were compared, it is important that we record some further results following its administration.

Glucose. In the great majority of cases in which symptoms occur as the result of insulin administration, the percentage of glucose in the blood is very close to 0.045 when definite convulsions supervene. When the fall in blood sugar is very rapid, however, the level of blood sugar may be considerably below 0.045 per cent when convulsions appear, a condition of collapse and coma being commonly observed in such cases, with only mild convulsions. This is more commonly the case when the animals have been starved for some time prior to giving insulin. Occasionally symptoms develop at a blood sugar concentration that is distinctly higher (0.055 per cent), but this is unusual.

When 2 grams of glucose per kilogram body weight, dissolved in 7 or 8 cc. of water, are given subcutaneously, the rabbit, which before had been either throwing itself violently about on the floor or else lying stretched out in a semi-conscious condition, in from 2 to 5 minutes

after the injection, moves unsteadily into a sitting posture with its head drooping. It often remains more or less drowsy for some time, although it may be stimulated to move about the room, which it usually does in a perfectly normal fashion. Sometimes during recovery, especially when convulsions have been severe, the animal may behave in an excited fashion, bumping into objects in its path apparently because it is entirely unconscious of their presence. The blood sugar determined within 15 minutes after the glucose injection has always been found to be considerably above 0.045 per cent. Frequently within a varying period after the injection, the rabbit has a second series of convulsions, accompanied as before by marked hypoglycemia. These may again be antidoted by injections of glucose. The glucose may be added to the blood stream in other ways than by subcutaneous injection; thus by oral administration of such sugars as become hydrolyzed to glucose during digestion and by exciting increased hepatic glycogenolysis by the administration of epinephrin (J. B. Collip). It is by the former of these methods that premonitory signs of hypoglycemic symptoms following insulin can be warded off in man. The latter method (epinephrin) is found most useful for the treatment of unconscious (comatose) patients, unable to take sugar by mouth, and in whom the circulatory conditions are such as to cause delay in the subcutaneous absorption of glucose.

A few animals recover spontaneously from the convulsions but the large majority, if untreated by glucose, ultimately succumb. Following the subcutaneous injection of glucose evidence of recovery is usually very rapid (1 to 2 minutes) although it may take 15 minutes for the complete disappearance of symptoms; secondary convulsions may supervene, but not within several hours with average doses of insulin. Recovery often follows injections of glucose even after the symptoms have lasted until the animal is no longer showing any convulsions but is in deep coma with very shallow often periodic breathing, and is almost moribund. Under such conditions, the first sign of recovery is the recurrence of convulsions. In the deeply comatose condition referred to it has on several occasions been impossible to detect any reducing substance in the blood by the Shaffer Hartman method. With this mode of behavior as our criterion, we may now proceed to consider the experiments in which other substances than glucose were injected.

Pentoses. The observations in which *pentoses* were injected were all perfectly definite in showing that these sugars are incapable of relieving the symptoms associated with hypoglycemia. That they were

absorbed into the blood is shown in the case of arabinose at least by the marked increase in blood sugar.

Arabinose

Rabbit, weight 2.6 kgm.

9:50 a.m. Blood sugar 0.108 per cent
10:10 a.m. 4.0 cc. insulin injected
2:30 p.m. Blood sugar 0.047 per cent
2:55 p.m. 3.0 cc. insulin
4:10 p.m. Severe convulsions—4.0 grams arabinose in 15 cc. water injected
4:30 p.m. Animal showed slight improvement, could support itself unsteadily if placed in sitting posture. Blood sugar 0.112 per cent. Condition gradually became worse and at
4:55 p.m. severe convulsions occured and respirations ceased. Artificial respiration; 4.0 grams glucose injected
5:15 p.m. Condition much improved—Given 4 more grams glucose
5:40 p.m. Blood sugar 0.216 per cent. Animal apparently well, but there were jerky movements of head and front limbs
Found dead next day

Arabinose

Rabbit, weight 3.0 kgm.

9:55 a.m. Blood sugar 0.149 per cent
10:00 a.m. 3 cc. insulin injected
2:10 p.m. Blood sugar 0.070 per cent
2:50 p.m. 2 cc. insulin injected
6:55 p.m. Severe convulsions—4 grams arabinose subcutaneously
7:00 p.m. Condition became slightly improved as in preceding experiment, but gradually became worse until convulsions occurred again at
7:29 p.m. when 4 grams glucose were injected
8:00 p.m. Condition apparently normal

Xylose

Rabbit, weight 1.5 kgm.

9:40 a.m. Blood sugar—0.150 per cent
9:45 a.m. Insulin injected
2:00 p.m. Blood sugar—0.052 per cent
2:50 p.m. 2 cc. insulin
4:00 p.m. Convulsions occurred. Blood sugar—0.033 per cent
4:20 p.m. Severe convulsions, 4 grams xylose injected subcutaneously
4:30 p.m. Severe convulsions followed by collapse and cessation of respiration; 4 grams glucose injected—artificial respiration
4:37 p.m. Partial recovery
4:45 p.m. Complete recovery

These results show that although it raises the blood sugar, arabinose cannot alleviate the symptoms, neither can xylose, but in this case it is not certain that absorption into the blood had occurred since there was no determination of the percentage of the blood sugar.

MONOSACCHARIDE HEXOSES. With the available sugars of this group —galactose and levulose—a temporary recovery of the symptoms was sometimes observed so that more experiments were necessary than in the case of the pentoses. Typical results are recorded.

Galactose

(1) Rabbit, weight 2.6 kgm.

8:30 a.m.	Blood sugar 0.130 per cent
8:35 a.m.	4 cc. insulin in injected
3:03 p.m.	Convulsions. Blood sugar—0.039 per cent
3:18 p.m.	Severe convulsions, 4 grams galactose injected
3:30 p.m.	Rabbit sitting up, apparently recovered
3:45 p.m.	Convulsions
3:46 p.m.	Comatose. Blood sugar—0.108 per cent
3:55 p.m.	Sitting up
4:30 p.m.	Convulsions
4:45 p.m.	Comatose. Blood sugar—0.080 per cent
	Dead next morning

(2) Rabbit, weight 1.95 kgm.

9:30 a.m.	Blood sugar—0.162 per cent
9:50 a.m.	4 cc. insulin injected
1:20 p.m.	2 cc. insulin injected
2:30 p.m.	Blood sugar—0.105 per cent
2:40 p.m.	2 cc. insulin
7:30 p.m.	4 cc. insulin
8:45 p.m.	Rabbit collapsed. Blood sugar—0.042 per cent
9:10 p.m.	4 grams galactose injected, condition unchanged
9:40 p.m.	Blood sugar—0.138 per cent, condition unchanged
9:50 p.m.	Can sit up
10:15 p.m.	Hopping around
10:30 p.m.	Collapse
10:55 p.m.	Convulsions. Blood sugar—0.203 per cent
11:03 p.m.	Sitting up, seems recovered
	Dead next morning

(3) Rabbit, weight unrecorded

9:30 a.m.	Blood sugar—0.113 per cent
9:40 a.m.	5 cc. insulin (skate)
10:40 a.m.	Violent convulsions. Blood sugar—0.042 per cent
10:45 a.m.	4 grams galactose injected

11:00 a.m. Lying in collapsed condition but when roused hops unsteadily for a few feet. Blood sugar—0.082 per cent
11:15 a.m. Occasionally tries to sit up
11:30 a.m. Hops about unsteadily. Blood sugar—0.112 per cent
12:25 p.m. Sitting up, chewing movements
12:35 p.m. Blood sugar 0.106 per cent
1:15–3:00 p.m. Condition unchanged, no convulsions
3:00 p.m. Blood sugar 0.036 per cent
3:50 p.m. Blood sugar 0.041 per cent, very drowsy
4:45 p.m. Blood sugar 0.056 per cent, very drowsy

These observations show that galactose can partially antidote the convulsions due to insulin. Its effect, in comparison with that of glucose, is however unsatisfactory, being delayed and the process of recovery usually interrupted by periods of relapse. This occurs even although the blood sugar stands at a high level. These relapses are likely to become more severe and in two of the observations with this sugar the animal was found dead on the morning following the experiment. In the remaining case relapses were not present and although the blood sugar did not recover nearly so well as in the others, the animal survived. Galactose has been given in several other cases with the same partially successful results. This conclusion agrees exactly with that of Mann and Magath on dogs showing hypoglycemia as a result of removal of the liver.

Levulose

(1) Rabbit, weight 2.6 kgm.

9:25 a.m. Blood sugar—0.146 per cent
10:00 a.m. 5 cc. insulin
1:00 p.m. Several convulsions
1:22 p.m. Convulsion. Blood sugar—0.036 per cent
1:25 p.m. 4 grams levulose injected
1:30 p.m. Rabbit able to sit up
1:40 p.m. Hopping about, apparently quite normal
2:00 p.m. Blood sugar—0.095 per cent
3:05 p.m. Blood sugar—0.103 per cent
7:40 p.m. Quite normal until this time when collapse occurred; 4 grams glucose injected with complete recovery within 15 minutes

(2) Rabbit, weight 2.25 kgm.

9:30 a.m. Blood sugar—0.125 per cent
9:50 a.m. 4 cc. insulin injected
1:40 p.m. Blood sugar 0.051 per cent
5:20 p.m. Blood sugar—0.039 per cent. Rabbit very hyperexcitable—seemed to recover as soon as sample was taken

7:15 p.m. 1 cc. insulin injected
7:50 p.m. Violent convulsion—after which rabbit seemed recovered
8:30 p.m. 1 cc. insulin injected
8:45 p.m. Violent convulsions—respirations ceased—given 4 grams levulose and artificial respiration for half a minute
8:53 p.m. Sitting up
9:03 p.m. Blood sugar—0.122 per cent—can hop about
10:20 p.m. Hopping about in a circle—1 gram levulose injected
11:30 p.m. Animal was apparently quite recovered
Dead next morning

(3) Rabbit, weight unrecorded

9:44 a.m. Blood sugar—0.133 per cent
9:45 a.m. 5 cc. insulin (skate)
10:50 a.m. Blood sugar—0.042 per cent
11:15 a.m. Convulsions
11:20 a.m. Blood sugar—0.022 per cent. Convulsions
11:28 a.m. 4 grams levulose
11:32 a.m. Convulsions. Blood sugar—0.089 per cent
11:42 a.m. Sitting up
11:48 a.m. Hopping about
12:05 p.m. Blood sugar—0.105 per cent
12:15 p.m. Convulsions
12:18 p.m. Blood sugar—0.099 per cent
12:20 p.m. 2 grams levulose injected
12:23 p.m. Severe convulsion
12:50 p.m. Hopping about
1:30 p.m. Lying on side
1:50 p.m. Convulsion. Blood sugar—0.092 per cent
2:00 p.m. Collapsed
2:35 p.m. 4 grams glucose
2:45 p.m. Hopping about in normal fashion
4:00 p.m. Normal, blood sugar—0.130 per cent

The animal was then killed and the subcutaneous tissue of the abdominal walls found to be very edematous. This tissue and the clear watery fluid exuding from it were collected and found to contain a very large amount of sugar.

(4) This observation was almost a counterpart of no. 3. Severe convulsions were observed at 0.035 per cent blood sugar. During an hour following 4 grams levulose there were temporary periods of recovery and the blood sugar rose to 0.094 per cent. Four grams glucose were then given with immediate and permanent recovery.

The results with levulose show that this sugar can *almost* be used as a substitute for glucose, the characteristic feature of its action (as shown typically in exper. 3) being that the convulsions readily relapse after an apparently prompt recovery following its injection. It must be remembered that these relapses may also occur some time after

glucose (2 grams per kilo) has been injected but in this case a further injection has almost invariably been found efficacious, which has not been found to be the case with levulose. With glucose also the recovery for some time following an injection is usually complete while with levulose the animal appears to be continually on the verge of convulsions. This result does not agree with those of Mann and Magath who did not find that injections of levulose had any beneficial effect on the similar symptoms of dogs rendered hypoglycemic by removal of the liver. We have frequently repeated the observations and are quite convinced of the temporary beneficial effect of levulose.

DISACCHARIDES: *Maltose*

(1) Rabbit, weight 2.0 kgm.

9:20 a.m. Blood sugar—0.139 per cent
9:50 a.m. 4 cc. insulin injected subcutaneously
12:40 p.m. Convulsion
12:42 p.m. Blood sugar—0.051 per cent—seems recovered
12:51 p.m. Severe convulsions—4 grams maltose subcutaneously
12:58 p.m. Collapsed
1:05 & 1:09 p.m. Convulsion
1:12 p.m. Sitting up
1:25 p.m. Blood sugar—0.165 per cent
1:27-2 p.m. Convulsions every few minutes with apparent recovery in between
2:10 p.m. Blood sugar—0.240 per cent
2:10–4:55 p.m. Alternate periods of convulsions and recovery till respirations ceased at 4:55 p.m.

(2) Rabbit, weight 2.1 kgm.

9:05 a.m. Blood sugar—0.123 per cent
9:15 a.m. 4 cc. insulin injected subcutaneously
12:00 m. Convulsions
12:20 p.m. Convulsion—4 grams maltose injected subcutaneously
12:35 p.m. Blood sugar—0.073 per cent—seems recovered
1:25 p.m. Blood sugar—0.119 per cent—sitting up
1:50 p.m. Rabbit is listless, but if disturbed is able to hop about
2:30 p.m. Blood sugar—0.109 per cent—condition unchanged
3:05 p.m. Comatose
3:10 p.m. Convulsions
3:15 p.m. Blood sugar—0.120 per cent—severe convulsion
3:18 p.m. Respirations ceased—4 grams maltose injected and artificial respiration maintained
3:20 p.m. Respirations returned
3:30 p.m. Animal died

The observations with maltose were repeated with the modification that the injections were made intravenously instead of subcutaneously.

Rabbit, weight 2 kgm.

9:50 a.m.	Blood sugar 0.130 per cent
10:10 a.m.	3 cc. insulin
12:05 p.m.	Hyperexcitable
1:20 p.m.	Blood sugar 0.051 per cent
2:00 p.m.	3 cc. more insulin
3:10 p.m.	Violent convulsion
3:25 p.m.	2 grams maltose in ear vein
3:55 p.m.	2 grams maltose
5:45 p.m.	Rabbit very drowsy but no convulsions
6:10 p.m.	Violent convulsion
6:40 p.m.	Died

Rabbit, weight 2.15 kgm.

9:57 a.m.	Blood sugar 0.125 per cent
10:11 a.m.	4.3 cc. insulin
1:30 p.m.	Blood sugar 0.077 per cent
2:03 p.m.	4.3 cc. more insulin
5:00 p.m.	Convulsions
5:25 p.m.	Convulsions. Sits up between attacks
5:35 p.m.	3 grams maltose into ear vein
5:40 p.m.	Lying in drowsy condition
6:00 p.m.	Appears much recovered
6:12 p.m.	1 gram maltose into ear vein
8:00 p.m.	Much recovered and moves about normally when disturbed
10:00 p.m.	Condition unchanged

Lactose

Rabbit, weight 2.3 kgm.

9:40 a.m.	Blood sugar—0.114 per cent
9:50 a.m.	4 cc. insulin injected
1:50 p.m.	Blood sugar—0.064 per cent
4:20 p.m.	Hyperexcitable
5:25 p.m.	Violent convulsions—4 grams lactose injected
5:29 p.m.	Blood sugar—0.047 per cent
5:35 p.m.	Animal comatose
5:48 p.m.	Blood sugar—0.089 per cent
6:00 p.m.	Comatose
7:10 p.m.	Dead

Sucrose

(1) Rabbit, weight 2.0 kgm.

9:45 a.m.	Blood sugar—0.112 per cent
11:25 a.m.	4 cc. insulin injected
7:35 p.m.	Blood sugar—0.038 per cent—hyperexcitable
8:10 p.m.	Blood sugar—0.041 per cent—severe convulsions
8:25 p.m.	Severe convulsions—4 grams sucrose injected

8:28 p.m. Sitting up
8:42 p.m. Convulsion
8:45 p.m. Blood sugar—0.042 per cent—severe convulsion
9:00 p.m. Sitting up
9:20 p.m. Blood sugar—0.059 per cent—convulsions
10:08 p.m. Severe convulsions— 4 grams glucose injected
10:15 p.m. Sitting up—can hop about
10:20 p.m. Blood sugar—0.116 per cent—complete recovery

(2) Rabbit, weight 2.1 kgm.

9:30 a.m. Blood sugar—0.123 per cent
9:50 a.m. 4 cc. insulin injected
3:06 p.m. Convulsion
3:26 p.m. Severe convulsion
3:45 p.m. Blood sugar—0.036 per cent—severe convulsions till at
3:50 p.m. 4 grams sucrose were injected—animal comatose
3:58 p.m. Convulsion
4:00 p.m. Comatose
4:20 p.m. Blood sugar—0.052 per cent—comatose
4:35 p.m. Severe convulsion
5:03 p.m. Respirations ceased—4 grams glucose and artificial respiration
5:15 p.m. Sitting up
5:35 p.m. Blood sugar—0.270 per cent—seems normal except that jaws move for considerable periods, at intervals
8:50 p.m. Comatose—3 grams glucose and levulose mixed
9:05 p.m. Sitting up unsteadily
3:30 p.m. Next day—convulsions and death

(3) Rabbit, weight 2.5 kgm.

8:50 a.m. Blood sugar—0.122 per cent
8:51 a.m. 4 cc. insulin injected
12:15 p.m. Severe convulsions—blood sugar—0.036 per cent
12:25 p.m. Convulsions—4 grams sucrose injected
12:30–32 Violent convulsions
12:33 p.m. Comatose
12:52 p.m. Convulsion—blood sugar—0.054 per cent
1:07 p.m. Convulsion
1:18 p.m. Convulsion—4 grams glucose injected
1:25 p.m. Sitting up—normal
4:15 p.m. Convulsions—dead next morning

The results with the disaccharides are interesting because they show that the extent to which hydrolysis may occur in the blood is not adequate even in the case of maltose to yield a sufficient concentration of glucose to antidote the convulsions, neither can any one of these sugars, by itself, perform this function. This result is rather unexpected in the case of maltose since it is commonly believed that the blood and tissues contain a considerable amount of maltase.[1]

[1] cf. Oppenheimer, Die Fermente, Leipzig, 3rd August, 1910.

In the experiments in which this sugar was injected either subcutaneously or intravenously the percentage of blood sugar became immediately raised but there was no decided alleviation of the symptoms, although a part of the increase in blood sugar was presumably due to glucose. When lactose was injected, the increase in blood sugar was not so marked as in the case of maltose and there was no evidence that the severity of the symptoms was in any degree abated. The less marked effect on the blood sugar may depend on slow absorption of lactose from the subcutaneous tissues and since there is no lactase in blood, none of the increase in reducing power which did occur can have been due to glucose. The results with sucrose show an insignificant increase in the blood sugar over the lowest percentages found about the time at which convulsions occurred, a result which would be expected since there is no sucrase in the blood of normal animals, and there was no alleviation of the symptoms. This occurred however within a few minutes of the injection of glucose although in two of the three animals of this group the symptoms ultimately reappeared and the animals died. This late effect is difficult to explain. It may depend on the considerable time (1 to 2 hours) the animals were in convulsions during which irreparable damage may have been done the nerve cells. In the one animal which survived however the convulsions lasted for nearly 2 hours.

The unsatisfactory action of maltose renders it practically certain that glycogen would have no effect in antidoting the symptoms. Nevertheless it was thought of interest to inject this substance in order to see whether there is a sufficient amount of glycogenase in the blood to cause an appreciable increase in the sugar content. In two such experiments the results were entirely negative both with regard to the symptoms and any change in the percentage of blood sugar. The negative character of these results may have been due to slow absorption because of the colloidal nature of glycogen.

The specific effect of glucose in alleviating the hypoglycemic symptoms offers an opportunity to determine whether non-carbohydrate substances might be synthesized in the body so as to produce either glucose or some other reducing sugar. If glucose were formed the hypoglycemic symptoms would be relieved and the blood sugar percentage raised; whereas if other sugars were formed the latter only would occur, as for example is the case with arabinose or maltose. Observations have so far been made using glycerol and sodium lactate (racemic). In one experiment the effect of ethyl alcohol was also tried.

Glycerol

Rabbit, weight 2.7 kgm.

9:25 a.m. Blood sugar—0.130 per cent
9:30 a.m. 4 cc. insulin
2:10 p.m. Convulsions
2:15 p.m. Convulsions
2:30 p.m. Blood sugar—0.054 per cent—rabbit seems recovered somewhat
2:45 p.m. Severe convulsions—10 cc. glycerol injected
1:10 p.m. Blood sugar—0.064 per cent—animal in a semi-conscious condition
1:40 p.m. Blood sugar—0.048 per cent—condition unchanged
2:20 p.m. Mild convulsion
2:25 p.m. Severe convulsion
2:40 p.m. Severe convulsion—4 grams glucose injected
2:50 p.m. Animal dead

Rabbit, weight 2.4 kgm.

8.40 a.m. Blood sugar—0.123 per cent
8:45 a.m. 4 cc. insulin
1:00 p.m. Blood sugar—0.033 per cent—convulsions
1:03 p.m. Comatose—50 cc. of 12 per cent alcohol injected subcutaneously
1:07 p.m. Violent convulsion
1:20 p.m. Semi-comatose
1:30 p.m. Blood sugar—0.018 per cent—comatose
2:30 p.m. Blood sugar—0.021 per cent—respiration suddenly ceased. Given 4 grams glucose and artificial respiration
2:35 p.m. Respirations returned—still comatose
4:50 p.m. Condition unchanged

Sodium lactate

Rabbit, weight 2.85 kgm.

9:20 a.m. Blood sugar—0.132 per cent
9:45 a.m. 2 cc. insulin
10:45 a.m. Blood sugar—0.060 per cent
12:00 a.m. 2 cc. insulin
2:25 p.m. Blood sugar—0.046 per cent—hyperexcitable
3:12 p.m. 1 cc. insulin
3:50 p.m. Convulsion—blood sugar—0.051 per cent
4:10 p.m. Severe convulsion—given 10 cc. of a solution of sodium lactate (containing 4 grams lactic acid) subcutaneously
4:20 p.m. Sitting up
4:30 p.m. Convulsion
4:35 p.m. Blood sugar—0.047 per cent
4:36 p.m. Convulsion
4:40 p.m. Convulsion—10 cc. sodium lactate solution (4 grams lactic acid) injected
4:55 p.m. Severe convulsions
5:05 p.m. Blood sugar—0.051 per cent—seems recovered slightly

5:15 p.m. Convulsion
5:50 p.m. Severe convulsion—4 grams glucose injected
6:00 p.m. Sitting up—seems normal
9:30 p.m. Convulsions again appeared
9:50 p.m. 4 grams glucose injected but animal died
9:52 p.m. Blood sugar—0.052 per cent—obtained from heart

(2) Rabbit, weight unrecorded

Normal blood sugar not taken
Blood sugar—0.035 per cent after insulin
1:37 p.m. 19 cc. Na lactate (4 grams lactic acid) injected subcutaneously
1:44 p.m. Severe convulsions
1:50 p.m. Blood sugar—0.055 per cent—animal seems recovered
2:00 p.m. Animal sitting up—quite listless
2:20 p.m. Blood sugar—0.097 per cent—condition unchanged
4:10 p.m. Able to hop about
10:30 p.m. Mild convulsion
10:45 p.m. Blood sugar—0.100 per cent—animal listless
Found dead next day

It is perfectly clear that neither glycerol nor alcohol has any effect either on the reducing power of the blood or on the symptoms. In the most carefully controlled observation in which sodium lactate was injected no increase in blood sugar occurred but in another experiment this seemed to be the case. One result of this nature cannot, however, be taken as conclusive evidence that a synthesis of lactic acid to glucose had taken place since the effect may be due to spontaneous recovery of the animal from insulin. Further observations on the effect of lactates are however indicated by these results.

Collip has observed that there is a decrease in the carbon dioxide carrying power of the blood and that acetone bodies often make their appearance in the urine of rabbits injected with insulin (3). Dixon and Pember in this laboratory have also noted marked hyperpnea accompanied by a rise in the respiratory quotient often to well over unity, in similarly treated normal dogs. Since these facts indicate a disturbance in the acid base equilibrium we have on several occasions injected solutions of bicarbonate or carbonate subcutaneously into rabbits showing characteristic symptoms but have never observed that they are thereby abated.

CONCLUSIONS

1. The only sugar which can definitely antidote the symptoms that accompany the hypoglycemia due to insulin is glucose. Even when the animal is moribund at the time of injection this sugar may bring about permanent recovery.

2. Levulose,[2] galactose and maltose may be followed by temporary slight improvement in the symptoms and they cause a marked increase in the blood sugar.

3. Arabinose, xylose, sucrose and lactose have no apparent effect on the symptoms although there may be an increase in the reducing power of the blood (e.g., with arabinose).

4. Sodium lactate, glycerol and alkalies have no effect on the symptoms.

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[2] It has since been found that mannose is almost as efficient as glucose in removing the symptoms.

VISUAL PERCEPTION AND THE RETINAL MOSAIC

I. Retinal Mean Local Sign—an Explanation of the Fineness of Binocular Perception of Distance

EMELIE E. ANDERSEN and FRANK W. WEYMOUTH

From the Laboratory of Physiology, Stanford University

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The testing of aviators during the world war has emphasized among other visual requirements the importance of binocular perception of distance (1), (3), (4) and it has recently been pointed out (1), (5) that proper binocular vision is an essential, though little recognized, requirement for drivers of automobiles and is also an important consideration in industrial and vocational fields. It is interesting to observe that in these cases the necessity for quicker and more accurate judgment of distance is an accompaniment of more rapid locomotion, thus paralleling the biological fact that binocular vision is more highly developed in those forms having the most active powers of motion, notably many of the birds and some of the more active mammals. Now that man has taken to the air, defects of binocular vision of little consequence in ordinary life have become vital matters.

The present study is an attempt to redetermine the threshold of binocular perception of distance among persons with normal vision. In the light of the exquisitely minute thresholds obtained and the more detailed knowledge of the histology and physiology of the retina now at our disposal, the theories hitherto advanced seem inadequate and we have found it necessary to reconsider the fundamental mechanism by which such surprisingly fine discrimination is accomplished.

Section I. The literature of binocular vision is voluminous but exact determinations of the threshold under specified conditions are few in number. The thresholds obtained by the earlier workers, moreover, were not so small as to raise the question here under consideration—how disparities of images amounting to less than the size of one visual element are to be perceived. Many discussions of the theory of binocular perception, therefore, do not need to be considered here. In

the following brief review only those papers giving experimental results or pertinent theories of importance are included.

Hering's careful work with his fall apparatus (7) constituted the earliest experimental results of importance. This method gave a threshold of $\frac{1}{5}\frac{1}{0}$ the distance from object to observer, which at the distance here used, 2 meters, would amount to 40 mm. This would not, of course, approach the size of the retinal elements; his general theories of binocular vision are too well known to require comment. He considers, however, that this method does not give the ultimate acuity of the "Raumsinn" which is more exactly measured by the displacement of the segments of a line that can be recognized. This view is discussed in the conclusions (p. 580).

Helmholtz (8) made a careful determination of his own binocular threshold using needles 0.5 mm. in diameter; this he found to correspond to a retinal displacement of 0.0044 mm. Since this agrees closely with the necessary separation of two lines distinguished monocularly and with the size assumed by him for the cones, the special problem of perception of differences less than a cone was not raised.

This is also true of the work of Greeff (9). Although, working with a modification of Hering's fall apparatus, he obtained thresholds somewhat smaller than Helmholtz, corresponding to distances of 0.002 or 0.003 mm. on the retina, these were still not below the size of the foveal cones.

Javel's extensive work on binocular vision need not be considered here; his experimental work did not give thresholds smaller than the authors just mentioned.

The present form of the question was brought up by the work of Bourdon and Stratton, who obtained thresholds which were not only below those of previous workers but obviously far smaller than the visual elements. Bourdon found that differences of only 5 seconds might form the basis for correct judgments of distance and with the displaced line obtained correspondingly low monocular thresholds. Stratton in dealing with the displaced line clearly recognized the problem and offered an explanation which we will discuss in a later section.

Within the past few years the threshold of binocular vision has been carefully determined by a number of workers whose results show a general agreement. Howard, for instance, working with a specially designed apparatus, obtained thresholds as low as 3.67 seconds and even 3.37 seconds. Though his experimental data seem rather more adequate, others—as Wells, Eaton, and Hazen—reached similar con-

clusions. None of these, however, has advanced any theory as to how these minute differences are perceived. Hartridge has recognized this problem and has offered an ingenious theory which will be considered in a following section.

The general situation, then, is that successive workers have obtained evidence of more and more delicate binocular perception which now clearly indicates that image-differences much less than one cone-diameter may form the basis of correct judgment. Some of the later workers have recognized this problem and have offered special theories to meet it.

SECTION II: EXPERIMENTAL PROCEDURE. *Description of apparatus.* Two pieces of apparatus were used in our experiments. After a considerable series of experiments with the first instrument, it was thought desirable to construct a new instrument which would eliminate certain objectionable features.

Constructed on the same principle as Bourdon's (6) apparatus, the first instrument consisted of three vertical black wires (corresponding to the needles of Bourdon) of 1 mm. diameter. The middle wire was so mounted upon a slide that it could be moved toward or away from the observer in respect to the two outer wires which remained stationary landmarks. The three vertical wires were stretched and anchored taut between wooden strips so as to form the fourth side of a square. Each lateral wire, though not movable in respect to the observer, was movable in respect to the middle wire.

This arrangement permitted the distance between the threads to be varied, yet left the lateral reference wires always in the same vertical plane and equidistant from the observer. White cardboard was used as the background against which the black wires were viewed. The subject was placed 2 meters in front of the wires and looked at the wires through an opening 10 cm. $\times$ 12 cm., for an exposure of about 3 seconds. For accurate experimentation this apparatus proved unsatisfactory. First, it was difficult of operation, thus necessitating a second person's services to record data. Second, the exposure time was only approximately uniform. Third, and perhaps most serious, was the fact that, though the wires were as taut as possible, they could not be accurately lined up in the same plane throughout their length. Lastly, the white background proved undesirable because it gave rise to shadows and confusing after-images interfering with judgment.

With the purpose of eliminating these imperfections and decreasing the sources of error in judgment, a new apparatus was designed and

constructed by the senior author. In place of the drop ball system of Hering (7) or the needles of Bourdon (6), three heavy uniform threads 0.5 mm. in diameter were employed. The lead bobs attached to these threads were immersed in a battery jar filled with heavy oil in order that the threads might be brought to a dead stop quickly after any shift in position.

Each of the outer standard threads was suspended from an adjustable block provided with a scale indicating the distance from the middle thread. This arrangement gave rapid, accurate and easy adjustment. As in the first apparatus, the middle thread was movable in respect to the observer, while the lateral threads were movable only in regard to the interval from the middle thread. In series B the outer threads were 4 cm. from the middle thread; and in series C the distance between the threads was 1.5 cm. The position of the middle thread with respect to these standard threads could likewise be determined by a millimeter scale. To facilitate the movements of the middle thread, which in the course of the experiments had to be changed frequently while the outer two threads remained stationary as standards, a lever was attached to the sliding block that carried the movable thread and to the far side of the frame of the apparatus. The frame which supported the apparatus, like all other parts, was painted a dead black, thus eliminating disturbance from reflected light and unequal illumination. Black cotton velvet was used as the background against which the white threads were viewed and was found to give no shadows or after-images.

A uniform time for observation of the threads was obtained by the use of a shutter. This consisted of a black cardboard disc from which a sector of 180° was removed, mounted so as to rotate before the apparatus. By means of a weight and a release a single revolution of the wheel was permitted at one time. To prevent the motions of the revolving wheel from confusing or distracting the attention of the observer a black cardboard screen was placed in front so as to hide it entirely from the observer. A slit in this screen 10 cm. high by 12 cm. wide permitted a view of the three suspended threads during each rotation of the wheel, while every other part of the apparatus was concealed. The rotation of the wheel was regulated to give 1.5 second exposure of the threads. Although this is a period permitting accommodation, Hering (7) and Howard (1) have shown by instantaneous exposure experiments that accommodation is not a necessary factor in binocular judgment of distance. As our experiments were made with the subject

at 2 meters, a distance at which accommodation changes would be extremely minute, we deemed the elimination of this factor unnecessary. It is to be noticed also that with the distance of observations (2 meters) and with the amount of movement of the thread limited to 6 mm., changes in apparent diameter would be too slight to form a basis for judgment. This contention is supported by the observations of Howard (1) in which the threshold for monocular perception not only required a movement several times that necessary for binocular perception but also the "reaction time to make monocular judgments is very slow, while binocular is instantaneous."

Further experiments, discussed in the following sections, demonstrate conclusively that the perception of distance under these conditions is binocular. In the course of the experiments it was found necessary to reduce the size of the 10 cm. slit through which the threads were viewed. This was accomplished by an adjustable screen permitting the use of apertures ranging from 10 cm. to 2 mm.

A strong uniform illumination was secured by placing the apparatus directly beneath a skylight. All parts except the three suspended threads were painted black to avoid reflected light. A head rest to prevent motion of the head was so placed that the subject's eyes would be exactly 2 meters distant from the standard threads.

In conducting the experiments the threads were first carefully adjusted to lie in the same plane and equidistant. In series A and B the distance from the middle thread to each of the lateral threads was 4 cm. In series C the distance of 1.5 cm. was used. The lateral threads remained stationary throughout a series of experiments to serve as a reference criterion for the judgment of position of the middle thread in respect to these standard threads.

After being instructed in the nature of the experiment the subject was asked through a series of trials to indicate when the middle thread seemed "nearer," "farther" or the "same" distance in relation to the standard threads. He was given a scale whereby his degree of certainty of judgment was recorded. For example, if the thread was set nearer the subject than the standards, a "near-one" reply indicated absolute certainty; a "near-two" was to mean he was still sure but "not willing to bet his life on it;" a "near-three" implied that he still thought he had some basis for judgment; but a "near-four" was a guess as far as the subject was concerned—the psychological guess, for on the average the "fours" did give more than a 33 per cent correctness that mere guessing permitted (33 per cent because of the three answers

possible). Hering as reported by Burch (10) holds that if judgment is but pure chance the ratio of right and wrong answers will be unity if a sufficient number of cases is taken, and that if it is not unity there must be some power of distinguishing distance.

Though every effort was made to carry out the experiment as accurately as possible, variations were naturally encountered. It was noted that there was much individual variation in the assignment of the four degrees of judgment. This was due in part to an absence of training in introspection of many of the subjects, but was found by all a matter of some difficulty. For this reason no extensive classification on the basis of degree of judgment has been attempted in this paper. The subjects were college students, interested in scientific research, hence excellent coöperation and willing seriousness were secured. The visual acuity and interocular distance of each subject were carefully determined.

Preliminary experimentation showed that a 6 mm. variation was sufficient for the outer limits of the movable thread, i.e., at 3 mm. beyond zero point the movable thread was readily judged as being "far," and likewise at 3 mm. in front of zero point as being "near" in relation to the observer.

For convenience in recording, the minus sign (—) was used to indicate that the thread was set nearer the observer than zero, and the plus sign (+) that it was further. The positions utilized in the observation were: —3 mm.; —2 mm.; —1mm.; 0; +1 mm.; +2 mm.; +3 mm., inclusive, but were offered in such order that no sequence deductions could be made. Ten sets of readings were taken at a sitting, making a total of 70 judgments for each subject. A "ready" signal was given before each trial. The length of a set of observations was not such as to cause any complaint of fatigue, and usually two or three sets of observations, could be taken at a sitting. Fatigue effects causing great irregularities in answers were met with on several occasions when observations were made at the end of a long day of hard work, or when the observer had had less than the normal amount of sleep.

Series A included work with 50 individuals, while series B and C work with 9 individuals with from 6 to 30 sets of observations on each.

SECTION III. PRESENTATION OF EXPERIMENTAL RESULTS. The results when plotted by the usual methods proved to be very uniform in most cases. The effect of applying the Phi Gamma corrections as developed by Urban (11) and Boring (12) was tried in the more important sets but gave very slight changes from the empirical data. From

the graphs the medians were determined, the thresholds, as is customary, being taken as the points on the "near" and "far" curves where 50 per cent of correct answers were obtained. Since these points were not usually symmetrically placed with regard to the instrumental zero, the "thresholds" as measured from this zero are often unequal. For this reason we have considered half the interval from the "near" to the "far" threshold as a measure of binocular acuity, and the "thresholds" for various experiments are so recorded, followed by the total interval (double threshold) in parenthesis.

On the old apparatus, when the separation of the wires was 4 cm., data (series A) comprising seventy tests per individual were obtained on fifty different subjects, giving an empirical threshold of 1.08 mm. (2.16 mm.), when corrected, 1.13 mm. (2.27 mm.). Included among these subjects were a few individuals with lowered visual acuity as well as a few astigmatic cases. These all gave higher thresholds than the subjects with normal vision. However, inaccuracies in the apparatus accounted for the general high threshold obtained.

The new instrument, built for accuracy and constructed to obtain absence of glare and after-images, was used under the same conditions—4 cm. between threads 10 cm. long. Better, more constant results were obtained and a decidedly lower limen, 0.71 mm. (1.43 mm.), or corrected, 0.70 mm. (1.41 mm.). These and some related thresholds together with the corresponding retinal displacements and their angular equivalents are given in table 1.

On this new apparatus only individuals with approximately normal eyesight were used, three of the subjects wearing correcting glasses to bring their vision to the normal level.

Fourteen sets of data (series B) from 9 individuals were obtained. One subject furnished two sets, another five complete sets, for each of six changes in size of the aperture and therefore in the length of the visible thread. This series likewise called for 70 trials on each individual. Thread lengths of 10 cm., 5 cm., 2 cm., 1 cm., 5 mm. and 2 mm., respectively, were used. This comprised the first series on the new instrument and is designated as series B in the discussion.

The second series of data on the new apparatus is identical in nature with the first set with the exception that the distance between threads was narrowed from 4 cm. down to 1.5 cm., and that but three subjects were used, each giving a series through the six varying sizes of apertures. This was called series C. In the following table are given the results of these two series.

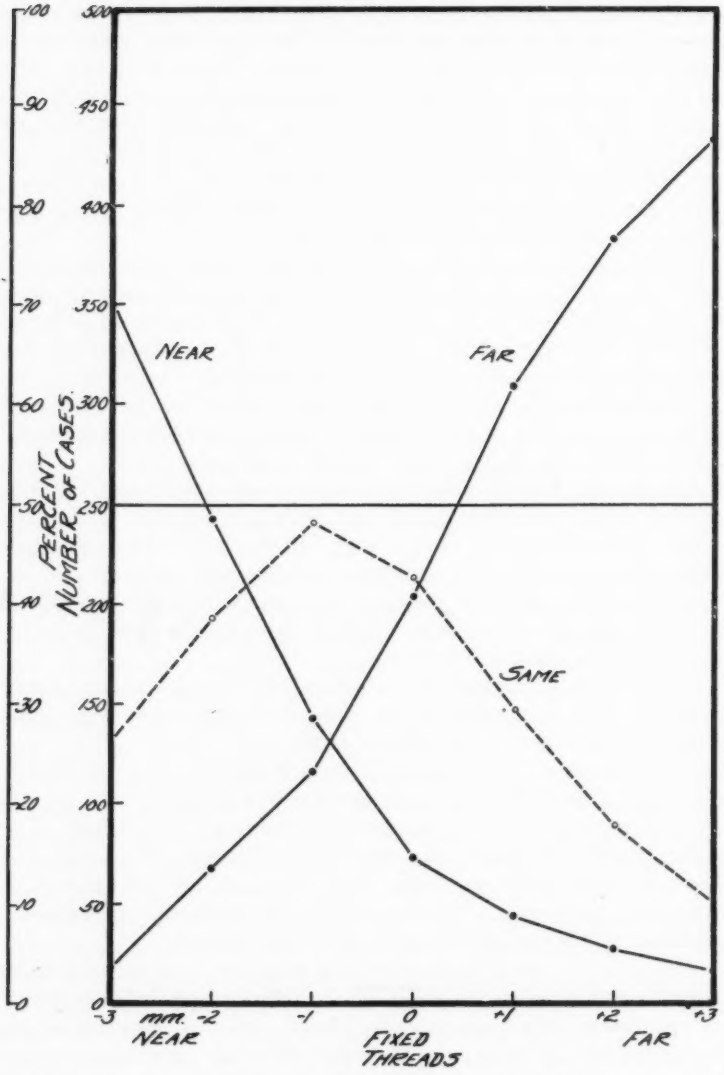

Fig. 1. Reports on position of movable thread. Series A; separation of threads, 4 cm.; "guesses" (no. 4) with "sames."

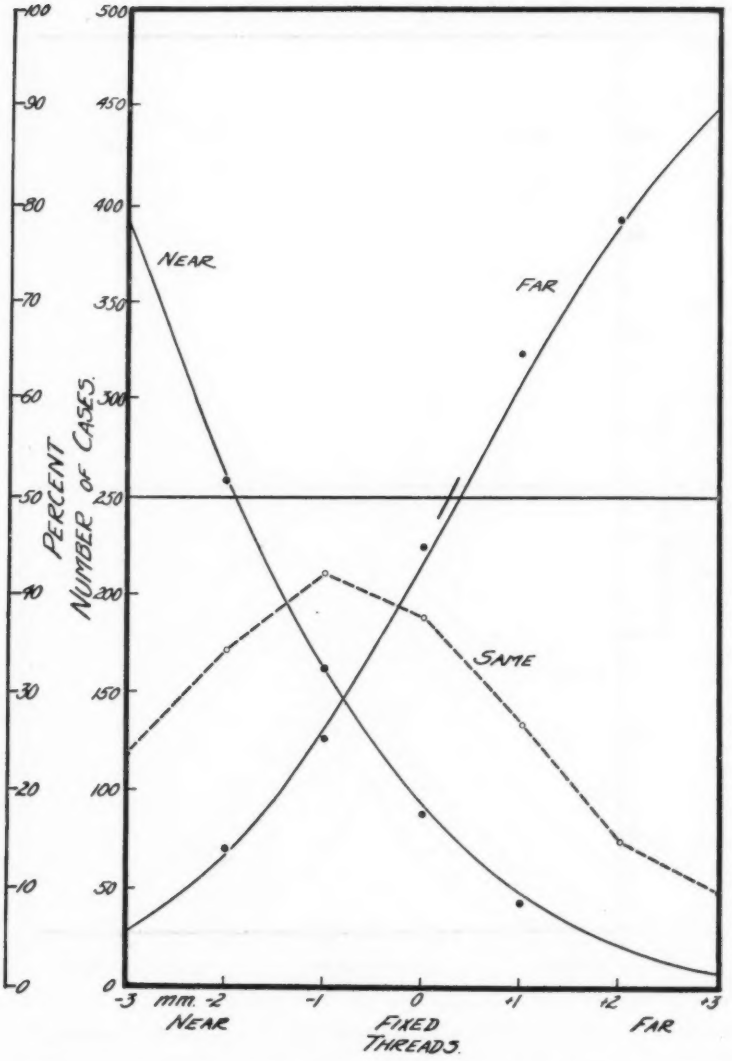

Fig. 2. Reports on position of movable thread. Series A; separation of threads, 4 cm.; "guesses" with "nears" and "fars."

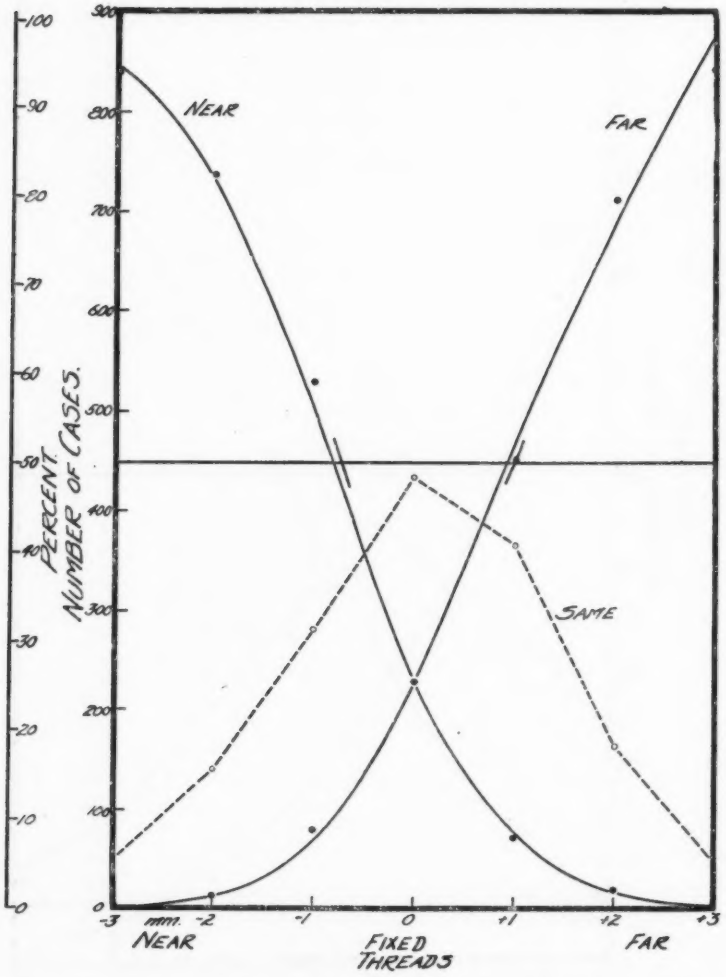

Fig. 3. Reports on position of movable thread. Series B; separation of threads, 4 cm.; "guesses" with "sames."

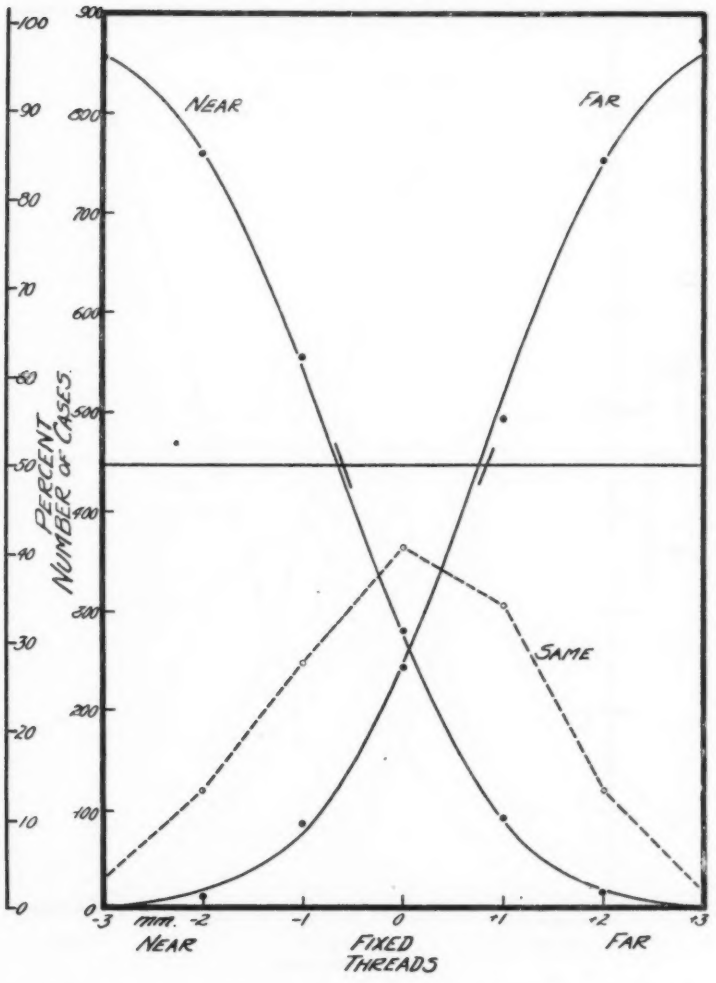

Fig. 4. Reports on position of movable thread. Series B; separation of threads, 4 cm.; "guesses" with "nears" and "fars."

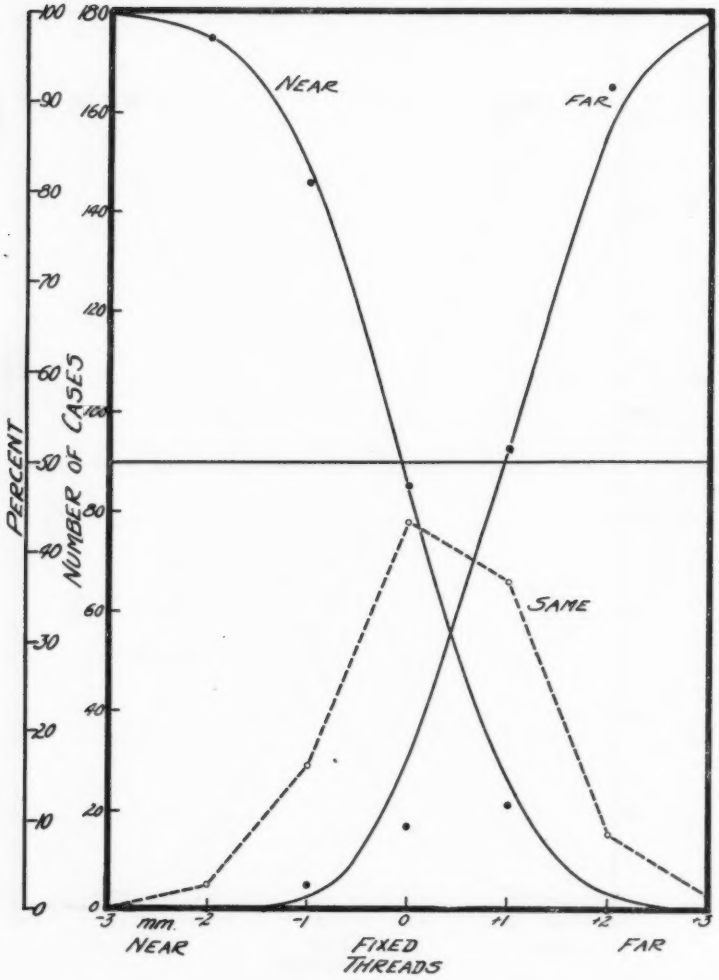

Fig. 5. Reports on position of movable thread. Series C; separation of threads, 1.5 cm.; "guesses" with "sames."

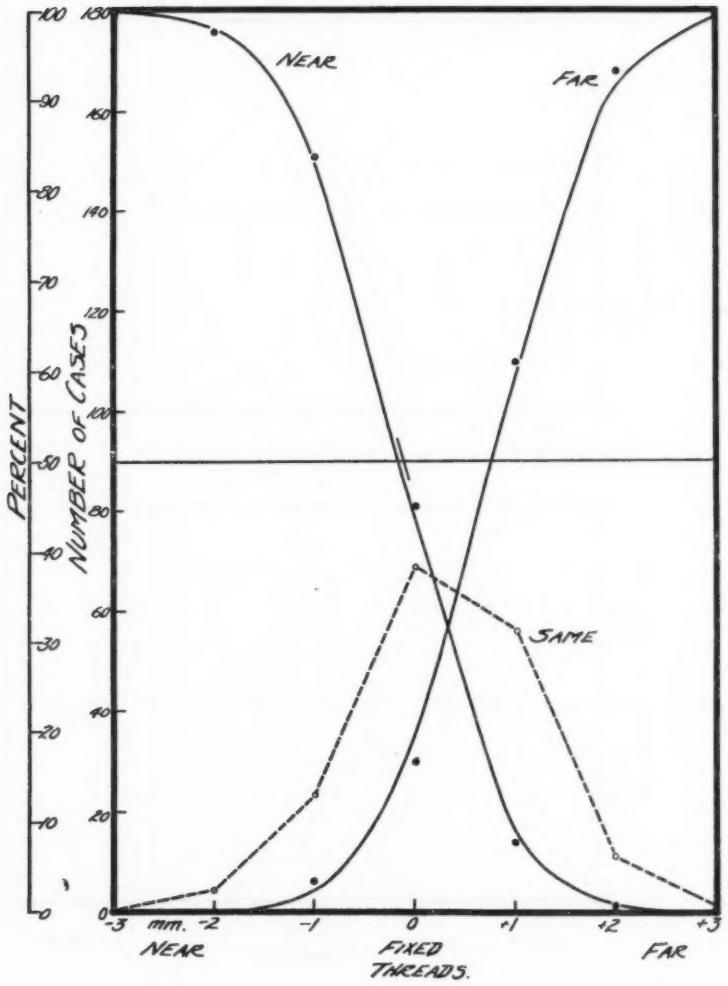

Fig. 6. Reports on position of movable thread. Series C; separation of threads, 1.5 cm.; "guesses" with "nears" and "fars."

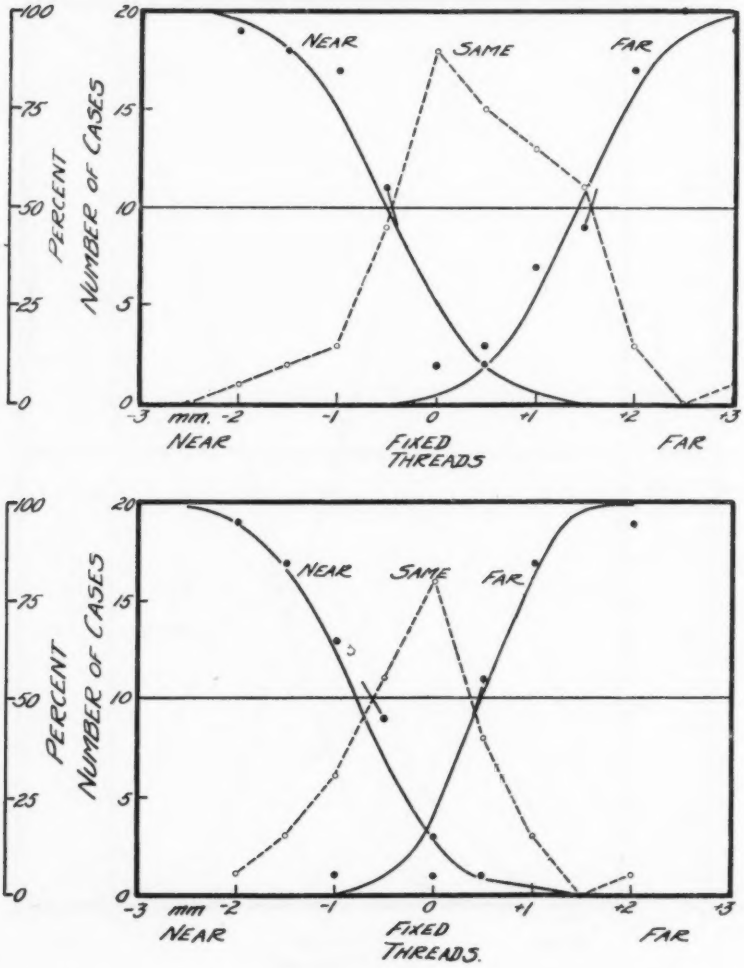

Fig. 7. Bourdon's data. Separation of threads in upper graph, 3.5 cm. in lower, 0.3 cm.

TABLE 1

Thresholds of binocular perception of distance. Threads 2 meters from observer, interocular distance of 63 mm. (average of observers) used in calculations. The number of the figure corresponding to each threshold is given. Bourdon's results are included for comparison.

SERIES	SEPARATION OF THREADS	LENGTH OF THREADS	GRADES OF JUDGMENTS INCLUDED	NUMBER OF OBSERVATIONS AT EACH STATION	NUMBER OF FIGURE	THRESHOLDS									
						Actual displacement of thread				Retinal displacement of image				Angular displacement	
						Double		Single		Double		Single		Double	Single
						E	$\phi\gamma$	E	$\phi\gamma$	E	$\phi\gamma$	E	$\phi\gamma$	E	E
	cm.	*cm.*				*mm.*	*mm.*	*mm.*	*mm.*	μ	μ	μ	μ	*seconds*	*seconds*
A 1	4	12	1, 2 and 3	500	1	2.50		1.25		0.621		0.310		8.14	4.06
A 2	4	12	1, 2, 3 and 4	500	2	2.16	2.27	1.08	1.13	0.536	0.563	0.267	0.279		
B 1	4	10 to 0.2	1, 2 and 3	900	3	1.73	1.73	0.86	0.86	0.428	0.428	0.213	0.213	5.60	2.79
B 2	4	10 to 0.2	1, 2, 3, and 4	900	4	1.43	1.41	0.71	0.70	0.354	0.349	0.176	0.173		
C 1	1.5	10 to 0.2	1, 2 and 3	180	5	1.03	1.03	0.51	0.51	0.225	0.225	0.125	0.125	3.34	1.64
C 2	1.5	10 to 0.2	1, 2, 3, and 4	180	6	0.88	0.93	0.44	0.46	0.218	0.230	0.108	0.113		
Bourdon	0.3	1		20	7	1.05	1.26	0.52	0.63	0.259	0.313	0.127	0.155	3.39	1.66
	3.5	1		20	7	2.00	1.95	1.00	0.97	0.496	0.483	0.247	0.240	6.50	3.24

That the results are dependent on binocular perception alone is indicated by a comparison with the thresholds obtained by Ghrist and Williams[1] from observations on a person who had lost one eye 3 years previously. That accommodation could play no part in thresholds of this size is indicated by the fact that this observer working with the same apparatus under identical conditions was unable to obtain with one eye a threshold within a range of 30 mm. on either side of zero, that is, the threshold exceeded ten times the range found sufficient for all normal binocular observations.

TABLE 2

Effect of length of thread on binocular perception. All grades of judgment included. Empirical curves used

SERIES	SEPARATION OF THREADS	LENGTH OF THREADS	NUMBER OF OBSERVATIONS AT EACH STATION	ACTUAL DISPLACEMENT OF THREADS		RETINAL DISPLACEMENT OF IMAGES				LENGTH OF RETINAL IMAGE
						DOUBLE		SINGLE		
				Double	Single	Linear	Angular	Linear	Angular	
	cm.	*cm.*		*mm.*	*mm.*	*μ*	*seconds*	*μ*	*seconds*	*μ*
B	4	10.0	140	1.6	0.80	0.395	5.17	0.157	2.59	787.5
	4	5.0	140	1.47	0.73	0.364	5.03	0.182	2.51	393.7
	4	2.0	140	1.1	0.55	0.272	3.56	0.136	1.78	157.5
	4	1.0	140	1.2	0.60	0.296	3.87	0.148	1.94	78.7
	4	0.5	140	1.4	0.70	0.346	3.53	0.173	2.26	39.3
	4	0.2	140	1.7	0.85	0.433	5.67	0.216	2.83	15.7
C	1.5	10.0	30	0.60	0.30	0.148	1.94	0.074	0.97	787.5
	1.5	5.0	30	0.79	0.39	0.194	2.54	0.097	1.27	393.7
	1.5	2.0	30	0.60	0.31	0.148	1.94	0.074	0.97	157.5
	1.5	1.0	30	0.87	0.43	0.214	2.80	0.107	1.40	78.7
	1.5	0.5	30	1.10	0.55	0.274	3.59	0.137	1.79	39.3
	1.5	0.2	30	1.23	0.61	0.303	3.97	0.151	1.98	15.7

That monocular parallax is not sufficiently delicate to contribute to the low thresholds under discussion is confirmed by the fact that when this one-eyed observer was allowed to move his head at will during the exposure of the threads, although the threshold was materially lowered it still remained four times (3.2 mm.) the size of the largest results quoted in table 1.

Inspection of table 2 reveals the fact that our lowest thresholds were obtained when a 2 cm. slit was employed. On plotting the results

[1] To D. G. Ghrist and T. B. Williams, the writers wish to express their indebtedness for permission to quote these unpublished results.

(fig. 8) we see that with thread lengths less than this all the data are in agreement and show a decrease in sensitivity with a decrease in length of image. With longer threads the results are less regular but show no higher sensitivity; this question is further discussed in a later section of this paper.

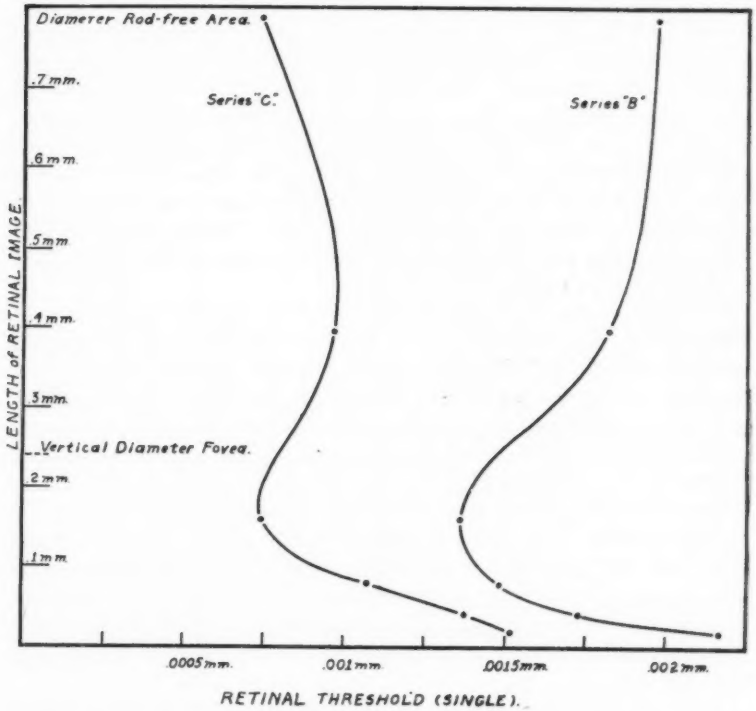

Fig. 8. Relation of length of retinal image to threshold. Separation of threads in series C, 1.5 cm., in series B, 4 cm.

Because of a statement made by W. Reber (13) that not until the twelfth year of life is binocular vision fully established, we tried to verify this by testing out four normal children, 6, 7, 8 and 11 years, respectively, but found that these children were capable of binocular perception of depth and able to compete with adults. Their thresholds are somewhat higher; however, much of this increase can be attributed to

the difficulty of holding the child's attention which is all too readily distracted to the mechanism operating the apparatus. The average threshold for these children was found to be 1.9 mm., less than the threshold obtained from adults on the old apparatus.

CONCLUSIONS

Our findings may briefly be summarized as a binocular perception of distance in which the retinal threshold falls far below the known size of the retinal elements. If we refer to the thresholds recorded in the last section (table 2, p. 576), we find that they range from 0.216 micron to 0.074 micron (0.000216 —0.000074 mm.). The cones of the fovea and macula which, as is well known, are smaller than elsewhere in the retina, range in diameter from 1.5 microns to 5 microns, according to the careful and extensive work of Fritsch (14). Shäfer (15) states that the diameter of the cones in the macula vary from 0.0015 (H. Müller) to 0.0054 mm. (Kolliker).

The largest threshold represents, therefore, slightly less than $\frac{1}{4}$ of the smallest value given for a cone, while if we take the smallest threshold and the largest values for the cone the fraction becomes less than $\frac{1}{10}$. Though our figures are smaller than those of most experimenters, due to careful control of conditions and to large series of observations, yet they represent no different order of magnitude and are supported by the results of Hering (7), Bourdon (6), and Howard (1), (2), (3).

Neither is the problem one of binocular vision alone. The accuracy of scale reading upon which depends the value of the vernier and the slide rule has been shown by Hering (7), Bourdon (6) and Stratton (17), in their experiments on the displaced line, to involve a threshold of the same order of magnitude as the binocular experiments, though the observations give practically the same result whether one or both eyes are used.

The problem of interest from the physiological side therefore becomes the harmonizing of the anatomical conditions with the experimental results when the magnitude of the perceived differences is clearly below that of the sensitive elements.

It may perhaps be objected that, though all the experiments are in agreement, diffraction effects render physically impossible the results as calculated by geometrical optics. It must be noted, however, that we are not dealing with images of the minute size of these thresholds but with shifts in the position of much larger images. It is of course true that the margin of the image is not the sharp one indicated by

the methods of geometrical optics, but is flanked by interference bands and distortions resulting from various optical defects, but this fact does not affect our argument. Suppose that we are dealing, as here, with an image of a light thread on a dark ground. Figure 9 shows the distribution of light intensity about the geometrical margin of an image (g) supposed to extend some distance to the left (adapted from Nutting, 16). Suppose under the conditions of the experiment an intensity represented by $a\ b$ is required to stimulate the retina, then the physiological margin of the image will fall at the point p. With a shift of

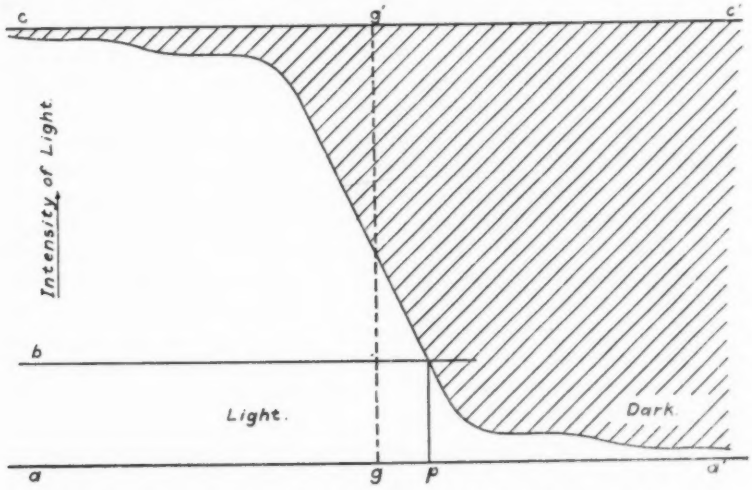

Fig. 9. Distribution of intensity of light about margin of image; gg', geometrical margin; ab, threshold intensity; p, physiological margin. Adapted from Nutting (16).

the geometrical margin in any direction on the retina, the point p where the intensity becomes liminal will shift to exactly the same extent and this will be true irrespective of the form of the intensity distribution. Since we are concerned only with this shift of position the methods of geometrical optics are entirely adequate. The questions involved in the discrimination of parallel lines, for instance, are very different and will not be considered here.

The problem as stated above has been recognized by all the workers who obtained these minute thresholds in their experiments. One solution was proposed by Hering (7) and later adopted by Bourdon (6)

to account for the findings in the displaced line. More recently Stratton (17) has proposed an entirely different explanation for the same phenomenon. Javal (18) and later Hartridge (19) have proposed theories of retinal stimulation to account for the fineness of binocular perception. These, as the more important, will be separately considered.

The conditions as conceived by Hering (7) and Bourdon (6) are represented by figure 10, reproduced from Bourdon (6). A shift of the lower segment of the line from right to left would under these conditions give rise to a sensation of a broken line, though the displacement is less than the diameter of a cone. With slight modification this could be applied to binocular vision.

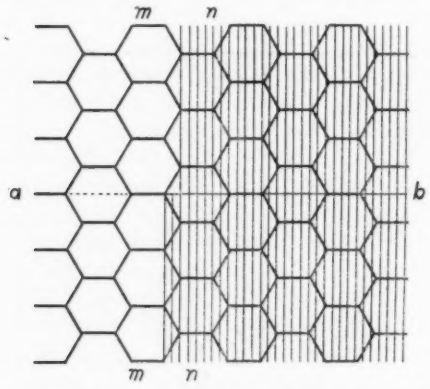

Fig. 10. Diagram to illustrate the theory of Hering and Bourdon. See text.

Neither Hering nor Bourdon would have proposed this theory today with the modern knowledge of the histology of the retina at their disposal. An examination of the figures of Fritsch (14), for instance, shows that the cones of the fovea have neither the hexagonal shape nor the regularity of ranked arrangement that this explanation requires. Furthermore, if the conditions of his diagram were exactly reproduced in the eye, a slight inclination of the line so that it did not parallel the rows of cones would completely abolish this fineness of perception. Bourdon (6) found, however, that the line might be tilted to any angle without materially affecting the threshold. Again, any eye movement, however slight, would make perception in this way extremely difficult if not impossible. This explanation must therefore be dismissed as entirely inadequate.

Stratton (17) has presented the theory that light not only affects those elements upon which it directly falls but also that the stimulation is diffused in all directions by responsive waves as evidenced by the fact of simultaneous contrast. "Now if C and C' (fig. 11) be adjacent elements, and b the boundary between them, then it seems not im-

probable that if a ray fall somewhere within the limits of C its effect upon C' will be different according as its point of incidence is nearer or farther from b. C' would be more intensely excited the nearer it fell to the limits of this cone; and, on the other hand, its effect upon C''' a neighbor on the opposite side of C would correspondingly decline. In such an event there would be a difference of nervous result with every change, however small, of the point of incidence instead of a change occurring only in case the stimulus passed to an entirely different cone. There would then be a difference of intensity of the diffusive effect, and consequently a change of 'local sign' whenever the difference of place of excitation is considerably less than the diameter of a single element."

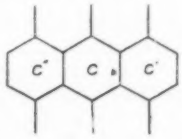

Fig. 11. Diagram from Stratton. See text.

This theory encounters equally formidable difficulties. In the first place it implies, though this is not absolutely essential, that the cones are arranged in straight rows. Secondly, the nice placing of an image so that it lies nearer one side of a cone than the other requires an accuracy of "fixation" that cannot occur. Lastly, and most seriously, it depends essentially on the assumption that different intensities of stimulation can be aroused in one cone and that these different intensities can be transmitted over the single connecting nerve fiber. While this cannot categorically be denied, it is clearly shown that, on the motor side, nerve conduction follows the "all or none" law (see for example Lucas, 20) and such graduation of intensities would be impossible. While this is not yet proven by direct experiment on the sensory side it would require clear proof to establish the contrary in view of the known similarity in most features between motor and sensory nerves. Such proof is not offered by Stratton.

For similar reasons the obvious explanation of fractional stimulation of a cone and fractional conduction in its nerve fiber, negativing, as it does, all anatomical knowledge of the retina and all knowledge of the physiology of nerve condition, may be at once dismissed as untenable.

Javel (18) has said that the movements of the eye muscles caused impulses to be sent to the brain which there are interpreted in terms of distance. But it is known that images which are formed on the retina for a short length of time (only 0.02 second) can be perceived in relief by binocular vision. The most serious difficulty, however, in ascribing the perceptions of minute differences either in binocular or monocular vision to the extrinsic muscles lies in the fact clearly shown by Dodge

(21) that irregular and unconscious movements continue during the most careful fixation, which since they are many times the angular magnitude of the thresholds here under consideration make it impossible that these are the result of kinesthetic sensations.

More recently, Hartridge (19) has proposed a different theory to account for the surprisingly low threshold in binocular vision.

This may be stated in his own words:

> To commence with, if depth depends on disparation it is clear that, when we perceive objects lying in different planes, we must subconsciously group them according as they fall on corresponding retinal points, or on points which are discrepant by one, two, three, or more cone-widths, and whether the discrepancy is crossed or uncrossed. The amount of the discrepancy must be some whole number of cone widths, because it is clearly impossible to stimulate half a cone with one impression and the other half of that same cone with a different one and obtain two distinct sensations. It is clear that space must be divided so far as stereoscopic vision is concerned into a number of concentric shells, the centers of which correspond with the position of the observer. Now the thickness of these shells can be readily calculated: at 1 meter they are found to be 2 mm. thick, at 10 meters 200 mm. thick, and at 100 meters 17 meters thick. If we are looking at a fixation mark 10 meters away, objects 100 mm. nearer to and 100 mm. farther from the observer will lie in the thickness of one and the same shell, and will therefore appear the same distance from the observer. Objects between 100 and 300 mm. nearer to the observer will lie in the shell corresponding to one cone discrepancy, and will therefore be appreciated as being at a different distance from the observer, appearing nearer if crossed and farther if uncrossed. The same reasoning applies to objects at other distances. If this calculation is correct it should be necessary to place objects more than 100 mm. from a fixation mark, which is itself placed at 10 meters, in order that a difference in the distance from the observer should be appreciated. Greeff found by experiment that 1/50th the distance of the fixation mark was necessary (i.e., 200 mm.), the observations being instantaneous ones. If time be allowed for prolonged observation, greater accuracy in the appreciation of distance is obtainable, because different points of fixation can be used. Suppose, for example, that two objects 20 mm. apart be examined at a distance of 10 meters, under instantaneous observation they will appear identical as described above; but if the examination be made more carefully it will be found that on fixating a point a mean distance of 100 mm. away from the objects, the distance between the two is suddenly appreciated because the demarcation between two shells now falls between them. It is in this way that the accuracy of extended observation becomes greater than that obtainable with instantaneous. The limit reached by experiment is stated to correspond to a displacement at the retina corresponding to the $\frac{1}{10}$ diameter of a cone.

While the conception of "shells" of distance represents a unique attempt to account for the fineness of binocular perception, it is evident that there are serious difficulties in the way of its acceptance. In the

first place it involves in equal measure with the theories of Hering (7) and Bourdon (6) the conception of accurately rowed cones and an image accurately parallel with these rows, both of which conditions the anatomical findings fail to confirm. Secondly, the imperfect fixation of the eye would here, as in the other cases, make the accurate placing of images on particular rows of cones impossible. In the third place, it could apply only to binocular vision, leaving the equally small monocular thresholds of the displaced line without an explanation. It would also fail to explain the effect on the threshold of altering the length of the line observed, while, as we shall see, this result strongly supports the hypothesis here put forward. It is thus clear that the well-established fact of a threshold below the size of a cone has received no adequate explanation.

One of the defects of all the current explanations is that they represent an unbiological point of view. No doubt a physicist considering a camera provided with a plate having a "grain" of 2 microns would dismiss as a physical impossibility a "resolving power" of any higher order. Yet an eye differs from a camera in at least two important regards, and it seems probable that these two differences, which from the physical point of view might readily be considered as defects, enable the eye to accomplish what the camera can not. In the first place, an absolute "fixation" of an object such as is necessary in a camera for a "time exposure" is impossible. Dodge (21) in his study of eye movements has shown that there are constant movements, slight in extent, yet far exceeding (15 seconds to 30 seconds) the thresholds here under consideration, both of the head and the eye, which must lead to a corresponding shifting of the image on the retina. Cobb (22) has recently emphasized that the normal method of retinal stimulation is a momentary one, and that even such imperfect fixation as can be maintained is an abnormal and unbiological use of the eye. It might carelessly be assumed that accurate fixation, as in the camera, were the ideal condition which the organism approximated as closely as possible and that the slight movements were unavoidable defects. But this imperfect fixation is associated with a peculiarity of the retina which brings us to the second difference between the eye and the camera. In a camera the effects of the image may accumulate on the plate and thus a time exposure record what fails to register with a momentary exposure. In the eye, continued exposure with even approximate fixation leads to speedy "fatigue," the "leveling down" of differences in intensity, and the development of troublesome after-images, so that

the first glance is the clearest. Under ordinary conditions the constant roving of the eye prevents more than a momentary stimulation of a portion of the retina with a particular portion of the image.

It is plain that such a conception as that of Hering (7) with its careful alignment of the image near the edge of a row of cones is an unbiological one; one for which the inaccuracies of fixation must figure as a defect. Let us see if these characteristics of the eye may not hold the secret of its surprising performances. In order to do this we must first get a clear conception of certain points regarding the local sign of the cones.

If, in the fovea, each cone has a distinct local sign we might expect certain appearances which we do not actually observe under ordinary conditions. For instance, the straight edge of a white card against a black ground should appear irregular, due to the fact that the rectilinear edge of the image is projected on an irregular mosaic of cones and the boundary of the area of stimulated cones must in consequence be irregular, just as the margin of a dark area in a half-tone is irregular if viewed under a lens. Such an appearance has been reported by some observers (23) in viewing parallel lines near the limits of visibility, but it is far from clear to most persons and certainly not as prominent a feature of vision as might be expected. That it is not so, probably depends on two main factors which we will consider. What relation obtains between adjacent cones stimulated by a stationary image? Assuming that each cone in the fovea has a "one to one" nervous connection and therefore a distinctive path to the brain—an assumption justified by our histological knowledge—there will be a possibility of a distinctive representation in consciousness, or local sign, for each one. The image of a straight line falling on one of two adjacent cones will usually stimulate both simultaneously. As a consequence the two cones will be linked together in experience and the local sign of each will partake of the nature of the other. Each cone stands in this relationship with the five or six cones surrounding it, and thus a complex linkage of cones will obtain throughout the retina. Presumably as we leave the fovea we must substitute larger and larger groups of cones for the single cone of the preceding discussion but the picture will otherwise hold for the entire retina. Essentially such a relation between neighboring cones has been postulated by Dodge (21).

What influence will these mutually interrelated local signs have on the stationary image of a straight line? Consider the three cones *a*, b and *c* (fig. 12) part of the margin of the stimulated area of cones which, from their arrangement, will tend to give an irregular edge to the image.

Cones *a* and *b*, having related local signs, when thus stimulated simultaneously would give a sense of position which might be said to tend toward their common center of gravity, and the same would be true of *b* and *c*. The projection formed by *b* on the outline of the image would therefore be pulled in, so to speak, by the influence of the adjacent stimulated cones with a total effect on the line of a marked smoothing, so that the percept of the margin of the image would be "straighter" than the line connecting the center of the last stimulated cones in our diagram.

But we know that such a stationary image is a physiological impossibility. As we have said, the slight but constant movements of both head and eye combine to cause the image of the object "fixed" to shift slightly but constantly across the sensitive elements of the retina. What would be the effect of this motion? With any conceivable arrangement of the visual elements a shift of a linear image perpendicular to its length for a distance equal to the average distance from center to center of contiguous cones would cause the stimulation of an entire new rank of cones. This must follow, since the line would shift from its first position to a corresponding position on the next cone. A shift of one-half of this distance would, if the line were long enough to give a fair average, cause one-half of such a rank to be stimulated, and smaller fractions would give correspondingly smaller parts of a new rank.

Since this conception is crucial for the theory here advanced of the perception of small retina image-differences, it will be profitable to examine it somewhat more closely. In figure 12 is reproduced an actual arrangement of foveal cones (represented by the circles) copied from a photomicrograph by Fritsch (14, plate LXIII, fig. 213, here enlarged 1,500 diameters).

One-half the field is illuminated (*L-L*) and one-half dark (*D-D*), the line of separation or geometrical margin of the image lying

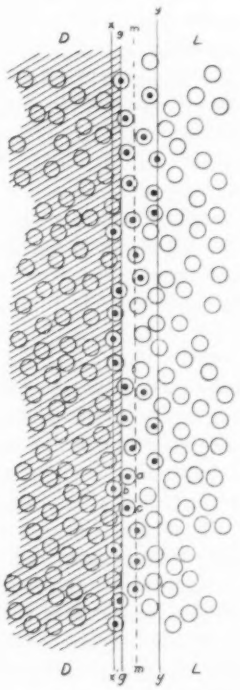

Fig. 12. Diagram of conditions at margin of stimulated area according to present view. See text.

along g-g'. If we assume any cone touched by light to be stimulated, all the unshaded cones will give rise to sensations and the physiological margin of the image will be represented by the line connecting the centers of the last stimulated cones (marked with heavy points) subject to the smoothing effect of their mutual effect just discussed. Should we consider that instead of being "touched," a cone must receive light on one half or any other fraction of its surface in order to be stimulated, the picture is only altered in this, that the relation of the geometrical margin and the physiological margin of the image will change by the corresponding fraction of a cone diameter. It is clear that though this physiological margin of the image (still thought of as stationary) is irregular, yet if we deal with a long enough line all points will lie within a strip having a width equal to the greatest interconal distance and standing always in the same relation to the geometrical margin of the image. It is also clear that we may substitute in the interests of simplicity "average interconal distance" for "greatest interconal distance" above.

Let us now consider the conditions when the image shifts, as it continually does, in relation to the retina. The physiological margin of the image will be a succession of such irregular lines, all points of which, however, will lie within the strip x-x', y-y' which must be thought of not as an area upon the retina but as maintaining a constant relation to the geometrical image.

Since in the long run equal numbers of points (centers of stimulated cones) will come to lie in all parts of the strip, the center of the strip, m–m', will represent the "center of gravity" of all the points stimulated and hence the local sign of the percept to which they give rise. The average or mean of these points, which determines the local sign of the straight line, is therefore not restricted to such units as interconal distance or cone diameter but may be accurate to a small fraction of these units just as the mean of a number of measurements made in inches may be accurate to a small fraction of an inch. Such a percept of position we would designate as "retinal mean local sign" to emphasize its derivation from the averaging of various factors among which we would include not only the many successive stimuli due to motion but also the mutual effect of adjacent elements and the comparison of the stimuli presented to the two eyes which we will presently discuss. Since both the "straightness" and the position of a straight line are complex judgments (even though unconsciously performed) the limits of accuracy are not set wholly by the fineness of the retinal mosaic

but by training and in general such neural processes as occur at a much higher level than the retina. The action of the moving image is also advantageous from another fact. What the retina perceives are really changes in intensity and hence the constant slight drifting of the eye, far from being a physiological defect, enables the retina to work at its highest efficiency, each cone thus being subjected to constant changes in intensity as the image shifts across it, while, as we have seen, the motion is also the basis of its fineness of perception in which it surpasses the limits set to the "resolving power" of a camera by the "grain" of the sensitive plate. This of course represents the biological development for, as Cobb has emphasized, prolonged fixation (even within physiological limits) is not normal with man and still less so with the lower animals. This effect is presumably not restricted to one retina but results also from the action of the two eyes. In view of the results of binocular experiments we must imagine that there exists a "corresponding point" relation between very small groups of cones if not between individual cones themselves. It is well known that even in ordinary illumination where the so-called "summation" of weak stimuli is not a factor, the acuity of two eyes is always greater than that of either, and it is probable that in the reading of fine type, for instance, where distance perception is not involved, the contours of the images are greatly improved by the superposition of the two images—a process of course occurring at a much higher level than the retina but working in harmony with the other factors that we have discussed.

So far we have been considering the rectification of the mental image by a manifold process of averaging; now we are in a position to apply the principle to one of the specific cases with which we started—the perception of a slight displacement of one of the segments of the broken line.

We have outlined the method by which a perception of a straight line may arise from many discrete stimuli and pointed out that its local sign will be the resultant or average of the positions of the component stimuli and like other averages accurate to a higher degree than any of the measures entering into its formation. If this occurs with each segment of the displaced line and the difference of the two local signs is great enough, the line will be perceived as discontinuous. How small this difference might be in terms of a cone-diameter cannot be stated *a priori*, and indeed, since, as we have seen, the comparison is between two percepts, it would be chimerical to expect to find in the physical configuration of the retina the final limits of its delicacy. From the

present experiments or those of Bourdon, it may be at least as small as $\frac{1}{10}$ of one cone-diameter.

The same principle may be applied to the present experiments in binocular vision. In figure 13 the arrangement of the images of the three threads in the two retinae are represented, though of course the differences are grossly exaggerated. Here a and b, a' and b' are the images of the fixed threads, while m and m' are those of the movable thread. The distances am and $a'm'$ may differ by only 0.4 to 0.148 micron or about $\frac{1}{5}$ to $\frac{1}{13}$ of a cone, and yet form the basis of a perception of distance. Here again this becomes intelligible as the result of a mean local sign which gives an average position accurate to a fraction of a cone. It is, however, surprising that so small a fraction can be appreciated in a comparison between the two eyes.

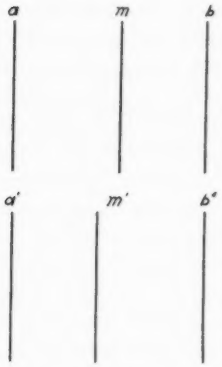

Fig. 13. Diagram of the images of the threads in the two retinae.

In our experiments it was found that the threshold was markedly affected by two factors: *1*, the separation of the images a, m and b; and *2*, by the length of these images. Are these effects in accord with the conception of mean local sign? The first would operate by bringing the lateral images into a more sensitive central region (with a separation of the threads of 4 cm. the lateral images fall outside the fovea) and by this and by the reduction of the distances to be compared would make judgment more accurate. This is in accord with the conception but furnishes no proof. The effect of varying the length of the image is more illuminating. By reference to figure 8 it will be seen that the sensitivity is at a maximum with an image of a 2 cm. thread, and that either longer or shorter images give larger thresholds. The second series, though based on less material, is in essential agreement with this. With the longer image—the stimulus was a 10 cm. thread—it was noticed that the answer was affected by the part of the line chosen for fixation and that by a marked elevation or depression of the visual line resulting from tilting the head forward or back the answer could be changed from "near" to "far." This undoubtedly came from the well-known effect of elevation or depression of the gaze on the tilting of the vertical retinal meridians, and hence on the form of the horopter. The larger thresh-

olds may therefore be ascribed to variations in the level fixed in the longer threads, a factor which was not anticipated and therefore not controlled.

What is the cause of the higher thresholds with the shorter images? At the point of maximum sensitivity the image was 0.1575 mm. long, corresponding roughly to 50 or 60 cones, a number large enough to give a fair average in the operation of the mean local sign.

The next larger image used, measured 0.394 mm., which exceeds the vertical diameter of the fovea(about 0.24 mm.). The ends of the images of the threads would therefore fall outside the fovea and further extension into these much less sensitive regions could not be expected to add appreciably to the delicacy of perception. With the shortest thread used the length of the image was reduced to 0.0157 mm., corresponding to five or six cones. This number is far from adequate for averaging. Thus while these results are intelligible on the basis of mean local sign, it is hard to see how the length of the image would be of importance in either the explanation put forward by Hering (7) or by Hartridge (19).

It will be seen that though the data at hand are in agreement with the factors postulated, there is no experimental evidence on certain points. This lack we hope to supply by certain work now in progress. It should be clearly understood, however, that by the term "retinal mean local sign" we intend to indicate that the sense of position is a complex percept. Should any of the factors here proposed prove to be unimportant in its genesis, or should others, not at present recognized, prove to be important, the essential features of this view will not be altered.

We may then conceive of the retina by a three-fold process of averaging—by mutually interacting local signs, by movement, and by combining the local signs of the two retinae—arriving at a mean local sign far exceeding in delicacy of perception any photographic plate of similar "grain" and giving judgments in tenths of cones when the cone might be expected to figure as the ultimate unit.

Several other interesting facts were brought to light. From experiments upon four children we found that at ages of from 6 to 11 years they were quite capable of perceiving distance binocularly. How much earlier this faculty develops we are unable to state, but it is probably a function that develops in early childhood and has reached a high degree of acuity at 6 years of age. These findings are contrary to those of Reber (3) who states that binocular stereoscopic vision is not always established before the twelfth year. He, however, presents no data.

We also found that those individuals with lowered visual acuity also showed impaired depth perception. It is highly probable that inaccurate judgment of relative distance and of speed is responsible for many of our motor accidents, as has been shown to be the case in certain of the accidents of aviation. In view of this, careful tests of binocular perception of distance, and, if necessary, correction of visual defects with regard to binocular vision might well be required of automobile drivers. We are in accord with what Howard has said on this question (1), (2), (3).

SUMMARY

1. Experimental redetermination of the threshold of binocular perception of depth shows that this may even exceed in delicacy the results obtained by Bourdon (6), Hering (7), Howard (1), (2), (3) and Stratton (17). However, the thresholds in all cases are of the same order of magnitude.

2. The thresholds obtained correspond to retinal displacements far less than one cone-diameter in all cases.

3. In the light of modern knowledge of the histology of the fovea and of nerve conduction, the theories heretofore advanced to account for these small thresholds are unsatisfactory.

4. A theory to account for this fineness of depth perception is here proposed:

We believe that the visual stimuli give rise to a complex percept of position which we propose to call *retinal mean local sign*. At least three factors contribute to this mean local sign: *1*, the mutual effect of adjacent retinal elements in either retina; *2*, the averaging of the successive stimulus patterns on each retinal mosaic caused by the constant slight eye-movements; and *3*, the combining of the two simultaneous stimulus patterns presented to the two eyes. As a result of these three factors the mean local sign (in the present case of the images of the threads) may be so accurate that a disparity between images of a fraction of a cone-diameter may be correctly perceived.

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APPENDIX

TABLE A1

Data on which the thresholds of table 1 are based

Station	Far	Near	Same	Total	Phi Gamma Far	Phi Gamma Near
SERIES A. 1. SEPARATION OF THREADS 4 CM. (ALL GRADES OF JUDGMENT RECORDED AS GIVEN)						
+3.0	434	17	49	500	447.0	7.6
+2.0	393	33	74	500	390.0	20.9
+1.0	323	43	134	500	310.0	47.2
0.0	225	87	188	500	216.0	93.8
−1.0	127	162	211	500	130.0	160.0
−2.0	70	258	172	500	66.0	258.0
−3.0	20	362	118	500	27.0	394.6
SERIES A. 2. (ALL "4" RESPONSES PLACED WITH "SAME")						
	432	16	52	500		
	384	27	89	500		
	309	44	147	500		
	204	73	213	500		
	116	143	241	500		
	67	240	193	500		
	18	350	132	500		
SERIES B. 1. SEPARATION OF THREADS 4 CM. (ALL GRADES OF JUDGMENT RECORDED AS GIVEN)						
+3.0	870	1	19	890	858.0	2.0
+2.0	752	19	119	890	750.0	18.0
+1.0	493	92	305	890	516.0	90.0
0.0	244	280	366	890	244.0	276.0
−1.0	87	555	248	890	78.0	547.0
−2.0	12	760	118	890	20.0	762.0
+3.0	4	859	27	890	1.0	860.0
SERIES B. 2. (ALL "4" RESPONSES PLACED WITH "SAME")						
	842	1	47	890	878.0	1.0
	708	19	163	890	690.0	15.0
	453	71	366	890	475.0	78.0
	227	230	433	890	225.0	225.0
	80	529	281	890	69.0	500.0
	13	737	140	890	13.0	728.0
	4	839	47	890	1.0	847.0
SERIES C. 1. SEPARATION OF THREADS 1.5 CM. (ALL GRADES OF JUDGMENT RECORDED AS GIVEN)						
+3.0	179	0	1	180	179.0	0.3
+2.0	168	1	11	180	165.0	1.0
+1.0	110	14	56	180	109.0	16.0
0.0	30	81	69	180	35.0	77.0
−1.0	6	151	23	180	4.0	150.0
−2.0	0	176	4	180	0.0	177.0
−3.0	0	180	0	180	0.0	180.0
SERIES C. 2						
	177	0	3	180	178.0	0.2
	165	0	15	180	158.0	3.0
	93	21	66	180	93.7	26.0
	17	85	78	180	25.0	84.0
	5	146	29	180	2.5	148.0
	0	175	5	180	0.07	175.0
	0	180	0	180	0.0	179.6
NO. 1. BOURDON, DISTANCE BETWEEN NEEDLES 5″—3 MM.						
+3.0						
+2.5						
+2.0	19	0	1	20	19.9	
+1.5	20	0	0	20	19.6	
+1.0	17	0	3	20	16.4	0.4
+0.5	11	1	8	20	10.3	0.8
0.0	3	1	16	20	4.0	2.9
−0.5	0	9	11	20	0.85	7.0
−1.0	1	13	6	20	0.09	12.2
−1.5	0	17	3	20	0.01	16.5
−2.0	0	19	1	20	0 0	18.9
−2.5					0.0	19.7
−3.0						
NO. 2. DISTANCE BETWEEN NEEDLES 1°—35 MM.						
	19	0	1	20	19.7	
	20	0	0	20	18.7	
	17	0	3	20	15.8	
	9	0	11	20	10.8	
	7	0	13	20	5.4	0.67
	2	3	15	20	1.8	1.95
	0	2	18	20	0.4	5.0
	0	11	9	20		10.0
	0	17	3	20		14.9
	0	18	2	20		18.15
	0	19	1	20		19.5
	0	20	0	20		
	0	20	0	20		

TABLE A2

Data on which thresholds of table 2 are based

SERIES B. SEPARATION OF THREADS 4 CM. (ALL GRADES OF JUDGMENT RECORDED AS GIVEN)

	Length of thread 10 cm.				Length of thread 1 cm.			
Station	Far	Near	Same	Total	Far	Near	Same	Total
+3 0	169	0	1	170	139	0	1	140
+2.0	152	2	16	170	123	3	14	140
+1.0	84	23	63	170	90	4	46	140
0 0	25	54	91	170	41	42	57	140
−1.0	3	127	40	170	11	86	43	140
−2.0	0	164	6	170	1	116	23	140
−3.0	0	170	0	170	0	137	3	140
	Length of thread 5 cm.				Length of thread 0 5 cm.			
+3 0	140	0	0	140	132	0	8	140
+2.0	126	0	14	140	111	4	25	140
+1.0	74	3	63	140	82	7	41	140
0.0	41	42	57	140	49	38	53	140
−1.0	8	91	41	140	24	73	43	140
−2.0	0	127	13	140	2	114	24	140
−3 0	0	140	0	140	0	136	4	140
	Length of thread 2 cm.				Length of thread 0 2 cm.			
+3 0	139	0	1	140	131	1	8	140
+2.0	122	1	17	140	109	5	26	140
+1.0	78	16	46	140	75	19	46	140
0 0	38	48	54	140	40	57	43	140
−1.0	9	105	26	140	26	72	42	140
−2 0	0	125	15	140	9	98	33	140
−3.0	0	140	0	140	0	131	9	140

SERIES C. SEPARATION OF THREADS 1 .5 CM. (ALL GRADES OF JUDGMENT RECORDED AS GIVEN)

	Length of thread 10 cm.				Length of thread 1 cm.			
+3.0	30	0	0	30	30	0	0	30
+2.0	27	0	3	30	29	0	1	30
+1.0	18	2	10	30	22	1	7	30
0.0	0	19	11	30	3	11	16	30
−1.0	0	27	3	30	0	26	4	30
−2.0	0	30	0	30	0	29	1	30
−3.0	0	30	0	30	0	30	0	30

TABLE A2—*Concluded*

SERIES C. SEPARATION OF THREADS 1.5 CM. (ALL GRADES OF JUDGMENT RECORDED AS GIVEN)

	Length of thread 5 cm.				Length of thread 0.5 cm.			
Station	Far	Near	Same	Total	Far	Near	Same	Total
+3.0	30	0	0	30	30	0	0	30
+2.0	30	0	0	30	29	0	1	30
+1.0	14	2	14	30	19	2	9	30
0.0	2	20	8	30	7	9	14	30
−1.0	0	30	0	30	1	22	7	30
−2.0	0	30	0	30	0	30	0	30
−3.0	0	30	0	30	0	30	0	30
	Length of thread 2 cm.				Length of thread 0.2 cm.			
+3.0	30	0	0	30	29	0	1	30
+2.0	28	0	2	30	25	1	4	30
+1.0	18	2	10	30	19	5	6	30
0.0	2	18	10	30	8	12	10	30
−1.0	0	29	1	30	5	17	10	30
−2.0	0	30	0	30	0	27	3	30
−3.0	0	30	0	30	0	30	0	30